Technical Document 3251
December 2011

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ADMINISTRATIVE INFORMATION

This report was prepared for the Navy's Environmental Sustainability Development to Integration by the Environmental Sciences Branch (Code 71752) of the Advanced Systems and Applied Sciences Division (Code 71700), SSC Pacific.

Released by	Under authority of
Charles Katz, Head	Martin Machniak, Head
Environmental Sciences Branch	Advanced Systems and Applied Sciences Division

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ABBREVIATIONS AND ACRONYMS

2-D	Two Dimensional
3-D	Three Dimensional
AA	Atomic Absorption
ACF	Advanced Chemical Fingerprinting
AFC	Antifouling Coatings
ARA	Antibiotic Resistance Analysis
ARCC	Average Rate of Correct Classification
BHVO	Basalt Hawaii Volcanic Observatory
BLM	Biotic Ligand Model
BMP	BESP Management Practice
BRAC	(Defense) Base Realignment and Closure (Act)
BST	Bacterial Source Tracking
CC	Chelex Column
CCC	Countercurrent of California
CCS	California Current System
CCSF	City and County of San Francisco
CD	Coefficient of Determination
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CH3D	Curvilinear Hydrodynamics in Three Dimensions
CLPP	Community Level Physiological Profiling
CNR-SW	Commander, Navy Region Southwest
COC	Contaminant of Concern
COPC	Contaminants of Potential Concern
CSIA	Compound Specific Isotopic Analysis
CSM	Conceptual Site Model
CSO	Combined Sewer Overflow
CWA	Clean Water Act
DDT	dichlorodiphenyltrichloroethane
DESI	Desorption Electrospray Ionization
DHS	(California) Department of Health Services
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
DQO	Data Quality Objectives
E. coli	Escheria coli
EBV	Epstein-Barr Virus
EcoRisk	Ecological Risk
ELISA	Enzyme Linked Immunosorbent Assay
EM (-1,2,…)	End-member (number 1, number 2, etc.)
ESTCP	Environmental Security Technology Certification Program
FIB	Fecal Indicator Bacteria
FISH	Fluorescence in situ Hybridization
FS	Feasibility Study
gal	gallon
GC	Gas Chromatography

GC-ECD	Gas Chromatography Electron Capture Detection
GC-MS	Gas Chromatography Mass Spectrometry
GFAA	Graphite Furnace Atomic Absorption
GIS	Geographical Information System
HBC	Hepatitus C
HBV	Hepatitus B
HCA	Hierarchical Cluster Analysis
HDPE	High-Density Polyethylene
HHV-8	Human Herpes Virus 8
HIV	Human Immunodefeciency Virus
HPLC	High Performance Liquid Chromatography
HPS	Hunters Point Shipyard
HPS	High Purtity Standard
HR-GCMS	High-Resolution GC-MS
HRGC/LRM-SIM	High-Resolution GC/Low Resolution MS in SIM Mode
HRGC/LRMS	High-Resolution GC/Low Resolution MS
HTLV	Human T-cell Leukemia/Lymphoma Virus
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IRMS	Isotope Ratio Mass Spectrometry
IUPAC	International Union of Pure and Applied Chemistry
Kg	kilogram
L	liter
m	meter
MAR	Multiple Antibiotic Resistance
MC-ICPMS	Multiple Collector Inductively Coupled Plasma Mass Spectrometry
MCMS-ICP-MS	Multiple Collector Magnetic Sector ICP-MS
MESC	Marine Environmental Survey Capability
mg	microgram
mil	million
mL	milliliter
MLST	Multi-Locus Sequence Typing
mm	micrometer
MPCD	Marine Pollution Control Device
MRA	Multiple Resistance Analysis
MS	Mass Spectrometry
MST	Microbial Source Tracking
NAS	Naval Air Station
NAVFAC	Naval Facilities
NDAA	National Defense Authorization Act
NESDI	Navy Environmental Sustainability Development to Integration
nmol	nanomole
NOAA	National Oceanic and Atmospheric Administration
NOAA S&T	NOAA Status and Trends
NOTW	Navy Owned Treatment Works
NOV	Notice of Violation
NPDES	National Pollutant Discharge Elimination System
NPS	Non-Point Source

NRC	National Research Council
PAH	Polyaromatic Hydrocarbon
PARCC	Precision, Accuracy, Representativeness, Completeness, and Comparability
PBMS	Performance Based Measurement System
PC1	Principal Component one
PC2	Principal Component two
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyl
PCR	Polymerase Chain Reaction
pCu	-log[Cu]
pH	-log[H]
POTW	Publicly Owned Treatment Works
ppb	part per billion
ppm	part per million
ppt	part per thousand
pptr	part per trillion
PRP	Principle Responsible Parties
PS	Point Source
PSA	Potentiometric Stripping Analysis
PSNS	Puget Sound Naval Shipyard
PSNS&IMF	Puget Sound Naval Shipyard and Intermediate Maintenance Facility
PST	Pollutant Source Tracking
PVA	Polytopic Vector Analysis
QA	Quality Assurance
QC	Quality Control
R^2	Coefficient Coefficient
RAPD	Randomly Amplified Polymorphic DNA
RCC	Rates for Correct Classification
REE	Rare Earth Elements
RI	Remedial Investigation
RNA	Ribonucleic Acid
RPD	Relative Percent Difference
rRNA	ribosomal RNA
RSC	Rapid Sediment Characterization
RT-PCR	Reverse Transcriptase PCR
RV ECOS	Research Vessel "Ecos"
SCCWRP	Southern California Coastal Water Research Project
SEAS	Spectrophotometric Elemental Analysis System
SERDP	Strategic Environmental Research and Development Program
SIM	Selected Ion Monitoring
SPAWAR	Space and Naval Warfare
SSC Pacific	Space and Naval Warfare Systems Center
SRM	Standard Reference Material
SRWQIS	Southern Regional Water Program
SSCPAC	Space and Naval Warfare Systems Center Pacific
SSO	Site Specific Objective
ST	Sequence Type

STGFAA	Stabilized Temperature Graphite Furnace Atomic Absorption
SWRCB	State Water Resources Control Board
t-RFLP	Terminal Restriction Fragment Length Polymorphism
TIMS	Thermal Ionization Mass Spectrometry
TMA	Trace Metal Analyzer
TMDL	Total Maximum Daily Load
TOC	Total Organic Carbon
TRIM2D	Tidal, Residual, and Intertidal Mudflats in Two Dimensions
UAA	Use Attainability Analysis
UNDS	Uniform National Discharge Standards
USA	United States of America
USEPA	United States Environmental Protection Agency
USEPA ORD	USEPA Office of Research and Development
UV	Ultraviolet
UWM	University of Wisconsin Madison
WQC	Water Quality Criteria
WQS	Water Quality Standards
yr	year

1. INTRODUCTION

1.1 PROBLEM STATEMENT

The nature and extent of anthropogenic contamination associated with Navy sources is often difficult to define or discern, particularly when water-bodies are shared with other industrial facilities and complex patterns of contaminant transport and deposition exist. As a result, sediments and water may contain potential contaminants of concern (COC) from both Navy and non-Navy point sources (PS) and non-point sources (NPS), which confounds assignment of responsibility for mitigation due to the complexities of such COC mixtures. In certain situations, traditional methods of concentration gradients and source concentration analysis alone cannot track COCs to their respective sources.

Attribution of contaminant loads can provide crucial information to the Navy in compliance issues. Some compliance issues are total maximum daily loads (TMDLs) and stormwater National Pollutant Discharge Elimination System (NPDES) permits. For example, Commander, Navy Region, Southwest (CNRSW) estimated a cost of $100 million to modify existing piping systems that currently discharge to the San Diego harbor so that the entire volume of a storm's first flush can be captured and treated. This requirement was included in a storm-water permit as a result of a TMDL.

Navy bases are subject to TDML requirements, which is a holistic approach to receiving water quality. A TMDL of the water body is determined by how much discharge of a pollutant the water and associated sediment can accept *from all sources* while still meeting the stated water (or sediment) criteria. TMDLs are required for waters that do not meet water quality standards and can be listed as impaired, or above the water quality standard, based on water column, sediment, tissue, or biological data. In the absence of defensible source term information, the Navy, often considered a high profile discharger by the civilian sector, can be held responsible for a disproportionate share of the source pollutant burden, even if that amount of source load did not originate from the Navy.

Current tools that the Navy uses to identify sources of contaminants include, but are not limited to, degradation and transport models, concentration gradients, Geographic Information System (GIS), storm-water and groundwater samplers, and sediment traps. However, any single technology or approach has significant limitations , which result in an inherent level of uncertainty. To minimize the uncertainty, one approach is to track a related constituent as an independent verification of pollution sources.

Recognizing and unraveling multiple sources of contamination typically requires more advanced chemical fingerprinting data than normally is acquired in a conventional Navy study. In addition, advanced chemical fingerprinting of large numbers of samples and multiple matrix types can be cost prohibitive. Thus, the need exists for a process by which naval facilities can cost-effectively collect the appropriate type and quantity of data needed to recognize and distinguish between different sources of contamination in sediments and water proximal to former or existing Navy facilities. This process was described in previous 0817 projects that focused specifically on polycyclic aromatic hydrocarbons(PAHs) (Stout et al., 2003), and a similar process is proposed here as an approach for various contaminants such as metals, microbes, and other organic contaminants polychlorinated biphenyls (PCBs).

The Navy TMDL Prioritization Report (NAVFAC, 2006) identified water bodies receiving Navy discharges that regulatory agencies have listed as impaired. NAVFAC then prioritized the TMDLs associated with those water bodies. Impositions of permit limits for

new pollutants, reductions in allowable discharge levels in existing permits, imposition of more stringent best management practices (BMPs), and restrictions on expansions of discharges are expected as TMDLs are implemented by regulatory agencies.

The Prioritization Report (NAFVAC, 2006) explains that each TMDL identified as having an impact on the Navy was assigned a priority of high, medium, or low. These priorities were based on several factors, including: TMDL schedules, whether the Navy is believed to be a significant contributor to the water-body impairment, whether the Navy discharge is from a point versus a non-point source, and whether the Navy installation is a high-profile facility in the water body.

This Prioritization Report (NAVFAC, 2006) summarizes 631 TMDL impacts to the Navy. Of the 631 TMDL impacts to the Navy, 81 are identified as high, 99 as medium, and 451 as low.

The Prioritization Report (NAVFAC, 2006) shows that the five most common priority impairments associated with TMDL impacts to the Navy are PCBs, dissolved oxygen, fecal coliforms, mercury, and nutrients. These five pollutant parameters are associated with approximately 30% of the TMDL impacts to the Navy. The next eight common priority impairments include chlordane, copper, dichlorodiphenyltrichlorethane (DDT), benthic impacts, dieldrin, bacteria, turbidity, and biological. Together with the top five impairment parameters, these 13 priority parameters are associated with almost half of the TMDL impacts to the Navy. Figure 1 shows these impairment parameters, the number of TMDLs associated with each, and the breakdown by priority category for each parameter.

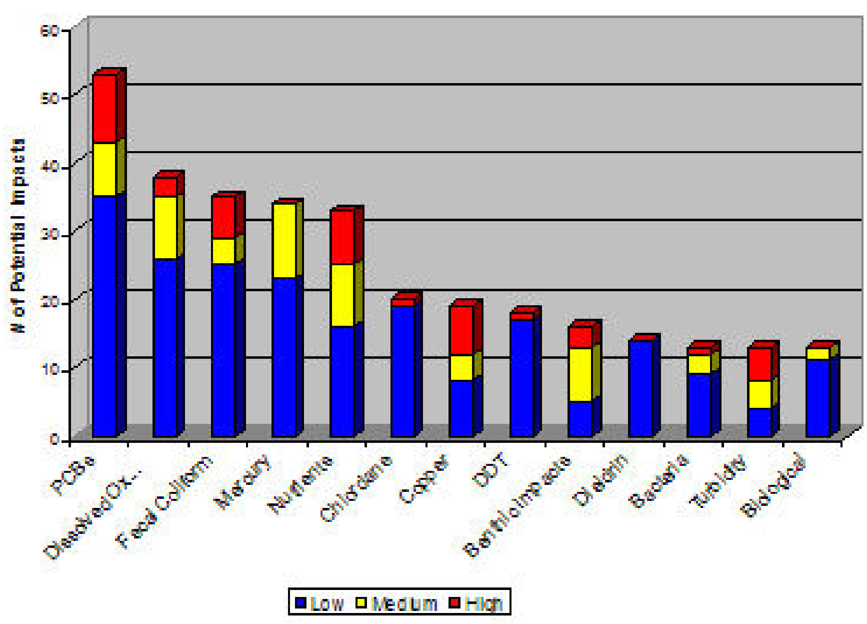


Figure 1. Top impairments based on number of potential TMDL impacts to the Navy.

In summary, the Prioritization Report states:

"• Most of the TMDLs impacting the Navy have yet to be developed or even scheduled for development. However, 120 potential TMDL impacts have been identified as being associated with TMDLs that have already been established. Of those 120, 34 have been assigned a ranking of "High" priority for the Navy.

• Currently, the top pollutants that will impact Navy installations via TMDLs are PCBS, dissolved oxygen, fecal coliforms, mercury, and nutrients. This list may change over time as states continue to identify additional water bodies requiring TMDLs.

• The majority of the TMDL impacts are from listings associated with assessment of the water column matrix, but there are a significant number of TMDL impacts associated with sediments, tissues, and ecology matrices."

1.2 OBJECTIVE

The objective of this project is to accurately quantify Navy contaminant loads by identifying, reviewing, demonstrating, and validating contaminant source tracking technologies that will provide a technical framework for Navy water program managers, enabling them to (1) attribute existing contamination loads to support compliance programs; (2) clearly understand the suite of tracking technologies currently available, their strengths and weaknesses, as well as how those technologies can be used to develop management decisions for compliance; (3) use this scientific approach and these tools to prevent arbitrary and burdensome regulatory decisions and actions that negatively impact the Navy.

1.3 GENERAL APPROACH TO THE PROBLEM

Robert Morrison in *Environmental Forensics* (2000) identified forensic techniques used to investigate the origin of contaminant release to include aerial photograph interpretation, underground storage tank corrosion models, literature reviews to identify the date when a chemical or additive became commercially available, association of a particular chemical with a manufacturing process, chemical profiling (fingerprinting), chemical degradation models, and contaminant transport modeling. This User's Guide will primarily focus on chemical fingerprinting, but will also touch on other key forensic techniques as needed.

1.4 BENEFITS TO THE NAVY

This product will provide the Navy with a means to attribute pollution loads for compliance programs so that a significant amount of money can be saved. The Navy will be able to identify base pollutant sources, which will in turn, help identify BMPs to reduce these sources.

2. TMDL SOURCE TRACKING FRAMEWORK

2.1 BACKGROUND

The basic formula for calculating a TMDL is TMDL=ΣWLA +ΣLA+MOS, where ΣWLA, or the sum of wasteload allocations, is equivalent to the total allowable loading from point sources, whereas ΣLA, or the sum of load allocations, is the total allowable loading from non-point sources. MOS is a "margin of safety" included to account for uncertainties and potential future growth in loading.

The following citation is the U.S. EPA's (1999) official definition of a TMDL. Number (4) below states that identification of source categories is part of the TMDL process. The U.S. EPA (1999) states:

"TMDLs are written plans and analyses established to ensure that the water body will attain and maintain water quality standards (existing uses, designated uses, numeric and narrative criteria and antidegradation requirements defined at 40 CFR 131) including consideration of reasonably foreseeable increases in pollutant loads. TMDLs must be established for water bodies on Part 1 of your list of impaired and threatened water bodies and must contain the following ten elements: (1) the name and geographic location of the impaired or threatened water body for which the TMDL is being established, (2) identification of the pollutant and quantification of the pollutant load that may be present in the water body and still allow attainment and maintenance of water quality standards, (3) identification of the amount or degree by which the pollutant load in the water body deviates from the target representing attainment or maintenance of water quality standards, (4) identification of source categories, source subcategories or individual sources of the pollutant for which wasteload and load allocations are being established, (5) wasteload allocations for pollutants from point sources, (6) load allocations for pollutants from nonpoint sources, (7) a margin of safety, (8) consideration of seasonal variation, (9) an allowance for future growth that accounts for reasonably foreseeable increases in pollutant loads, and (10) an implement-tation plan."

The project goal of this User's Guide is not to summarize the TMDL process, or discuss how to develop a TMDL, since the predecessor to this project (Navy Technical Guidance, 2004) has already accomplished those goals. For more information, please see that document or refer to other similar available documents provided by the U.S. EPA, Navy, and individual states.

The California State Water Resource Control Board (SWRCB) summarizes the regulatory options for addressing impaired waters in Figure 2. In most cases, it will somehow require reduction of pollutants. If the water quality standards are not being met because the applicable standards are not sufficient, an appropriate response may be to correct the standards through mechanisms, such as use attainability analysis (UAA), or a site-specific objective (SSO). The red box over "evaluate cause of impairment" is where this User's Guide would be applied to provide a framework and information for such an evaluation.

Figure 3 shows the EPA Guidance (U.S. EPA, 1999) on the 303(d) listing process and the TMDL Establishment process. The PST User's Framework would again be applied to source assessment.

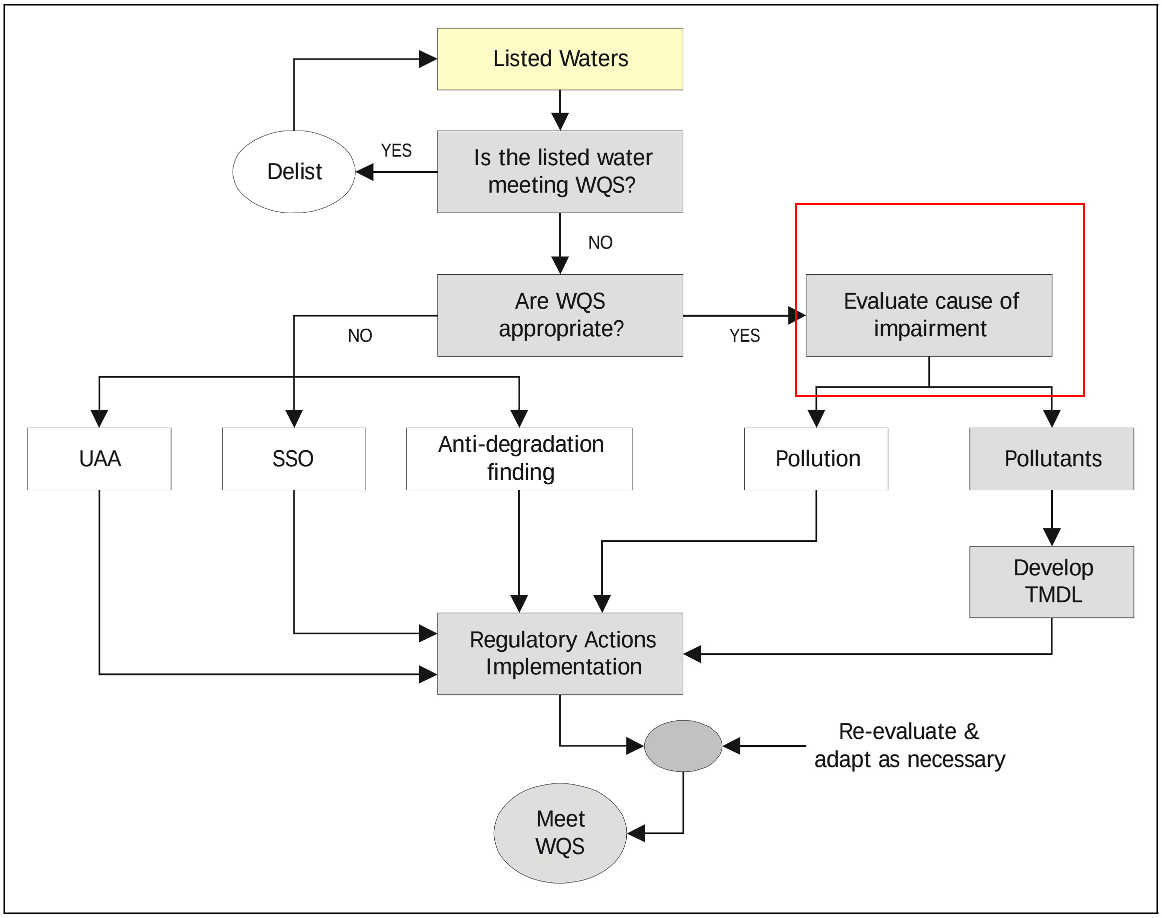


Figure 2. Conceptual diagram of California's water quality regulatory framework (Calif. SWRCB, 2003).

2.2 SOURCE TRACKING FRAMEWORK EXAMPLE

A TMDL source tracking framework that illustrates how this guidance can be used in a fingerprinting study, specifically within a TMDL project, is shown in Figure 4. This also adheres to the main steps of the data quality objectives (DQO) process. A separate framework summary is also presented for each major contaminant group (with the numbers representing chapters to find the respective information): framework overview of metals is shown in Figure 5 shows a framework overview of metals, Figure 6 shows a framework overview of microbes, and Figure 7 shows a framework overview of organics.

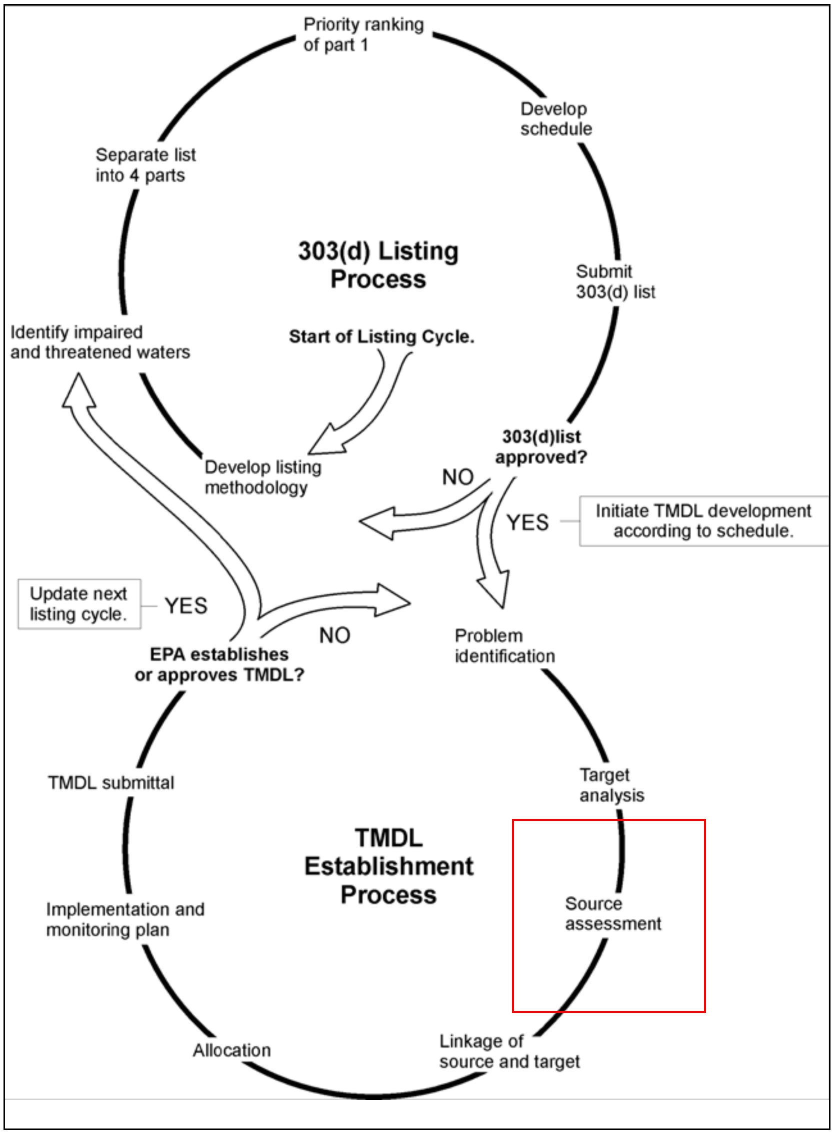


Figure 3. Components of the 303(d) listing and TMDL establishment process.

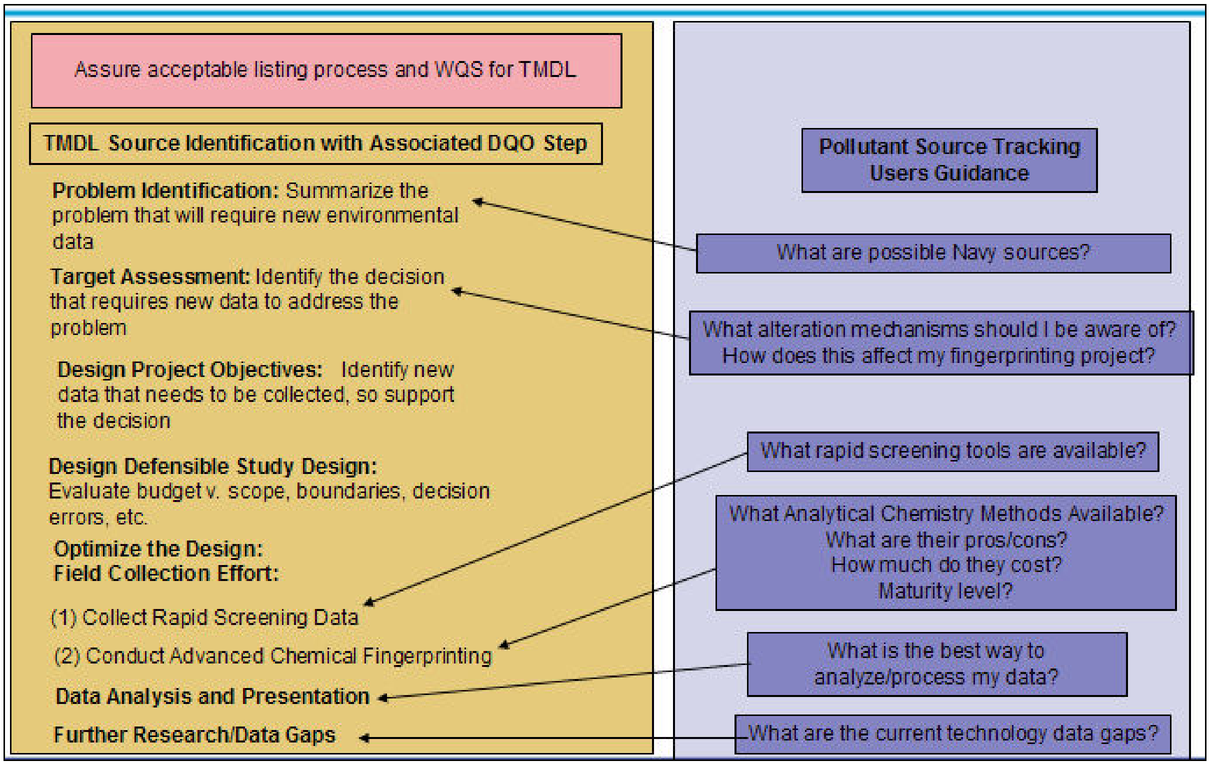


Figure 4. TMDL source tracking framework.

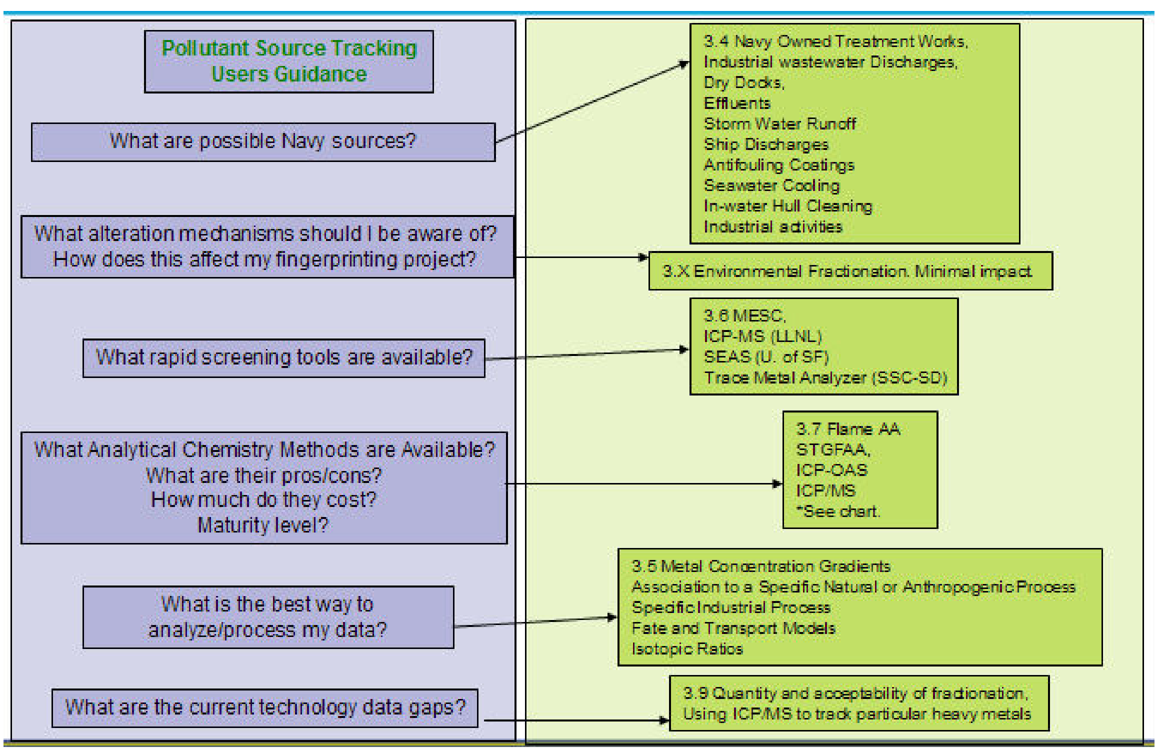


Figure 5. Source tracking framework overview of metals.

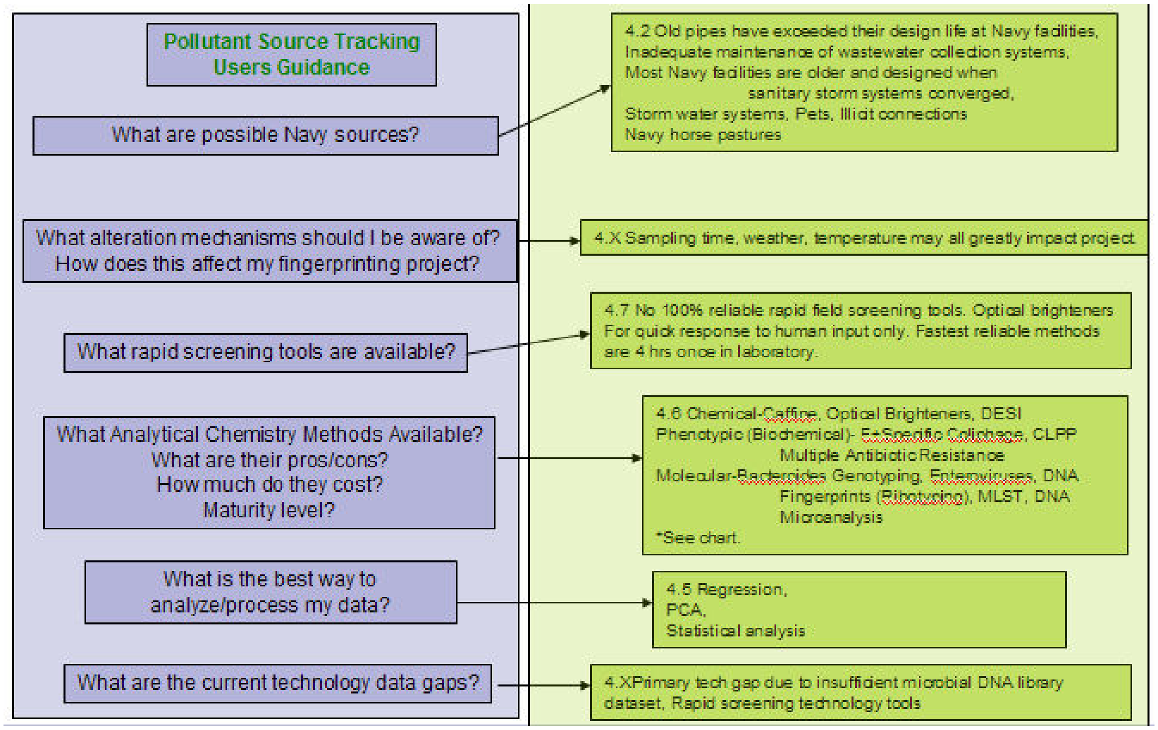


Figure 6. Source tracking framework overview of microbes.

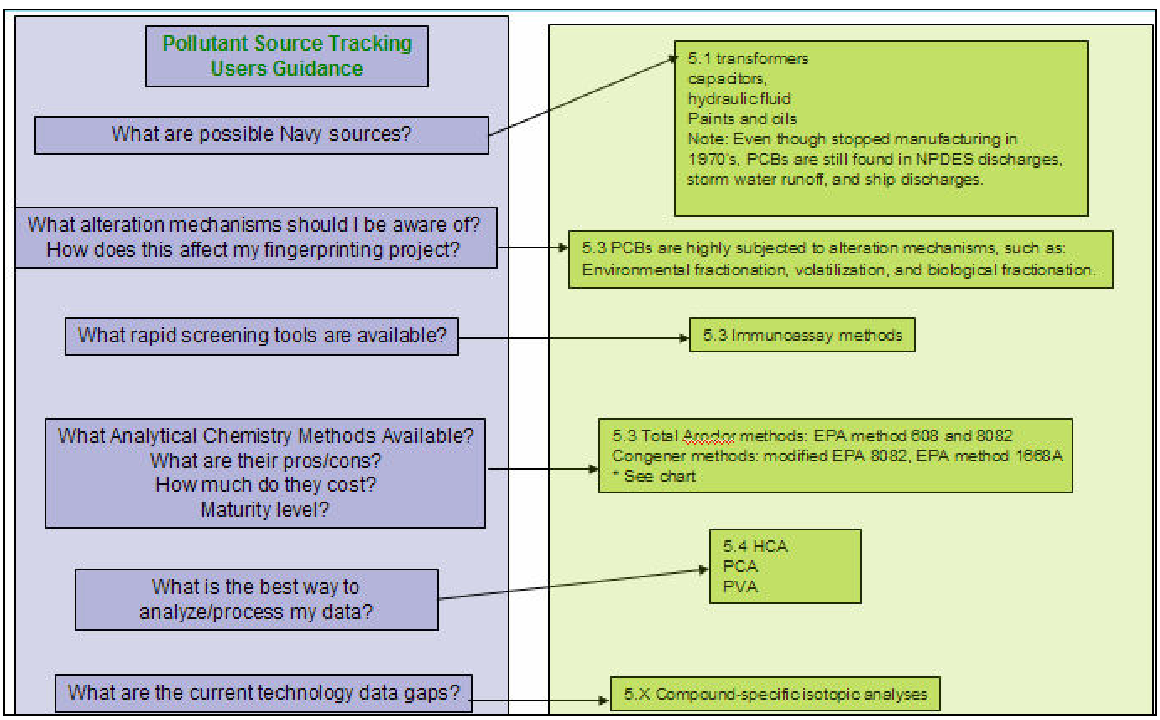


Figure 7. Source tracking framework overview of organics.

3. METAL SOURCE TRACKING

3.1 INTRODUCTION

Metals are used in a multitude of the industrial activities in the Department of Defense (DoD), and a result of this use is the release of metals to the environment. In general, if the level of release is relatively low, there is no effect to the environment; however, the nature of industrial activities can result in a release that could have a significant effect in the environment. There is a continued regulatory effort to control the effect of these metal releases in aquatic environments. This effort can be related to compliance activities by controlling metal concentrations at the point of discharge (i.e., NPDES and TMDLs) or cleanup activities, such as assigning liability to the different parties assumed responsible and enforcing remedial actions. This chapter focuses on the former, options for compliance of metal contamination in aquatic environments, and it is specifically directed to the assignation of responsibilities for ongoing and historical releases of metals to the aquatic environment.

Tracking and fingerprinting techniques for the identification of the original source of metals, or for the assignation of loads to different sources into aquatic environments are detailed. Most of the techniques presented here are directed at water column studies; however, these same techniques could be applied to sediment studies as well. The importance of working in the water column is illustrated in the Naval Facilities Engineering Command (NAVFAC) Total Maximum Daily Load (TMDL) Prioritization Report (NAVFAC, 2006), which indicates that 50% of the copper TMDLs are in the water matrix.

A suite of metals are of interest to DoD with respect to impairment. These metals include mercury and copper (Figure 1-1), and the examples of the tracking and fingerprinting techniques presented here can be applied to these and other metals of interest. The extent of the metal-related problems at Navy installations is illustrated in the list of Navy bases with metal impairments (NAVFAC, 2006) included in Appendix A. Information in this table indicates that 16 activities have copper impairments, 27 have mercury impairments, and 8 have zinc impairments. Not shown are three activities having only general metal impairments identified.

3.2 OVERVIEW OF METALS AS POLLUTANTS

Historically, environmental scientist have evaluated metal pollution in aquatic environments as function of total metal concentration. Therefore, these total metal concentrations were used to evaluate the potential environmental effects of metals. From the point of view of ecological health, metals can be classified in two groups, those metals that are essential nutrients at low concentrations but toxic at higher concentrations (copper and zinc), and the those metals that are toxic at the lowest concentration level (mercury and lead). Copper, for example, is a nutrient required for biological activities. At low concentrations, copper is required by marine algae for electron transport in photosynthesis (hymocyanin in crustaceans, and plastocyanin in marine algae that do not use the copper-free cytochrome, c_6) and by various enzyme systems (e.g., amine oxidase, cytochrome *c* oxidase, Gledhill *et al.*, 1997). Thus, at extremely low concentrations, requirements for copper can result in lower biological functioning (i.e., lower primary productivity; left-hand side of the curve in Figure 8. Once the copper concentration is above the threshold for productivity, there is a range of total copper concentration for optimal biological functioning, which is represented by the flat portion of the curve in Figure 8. However, at high total concentrations, copper becomes a toxicant, impeding biological functions, as indicated by the right-hand side of the curve in Figure 8.

Excess copper impacts many processes in the natural environment. It inhibits photosynthesis, disrupts electron transport in photosystem II, reduces pigment concentration, affects the permeability of the plasma membrane, induces loss of cations (particularly potassium), disrupts gametophyte development, inhibits nitrate reductase, restricts growth, affects cell motility, and affects the distribution of other compounds such as proteins, lipids, sterols, sterol esters, and free fatty acids (Gledhill *et al.*, 1997, and references therein). Therefore, copper is considered a pollutant when it is present at high total concentrations. In practice, total metal concentration is used as the regulatory tool for point sources, while dissolved concentration, which is considered more representative of copper toxicity, is used for regulation in water bodies (Protho Memo, U.S. EPA, 1993).

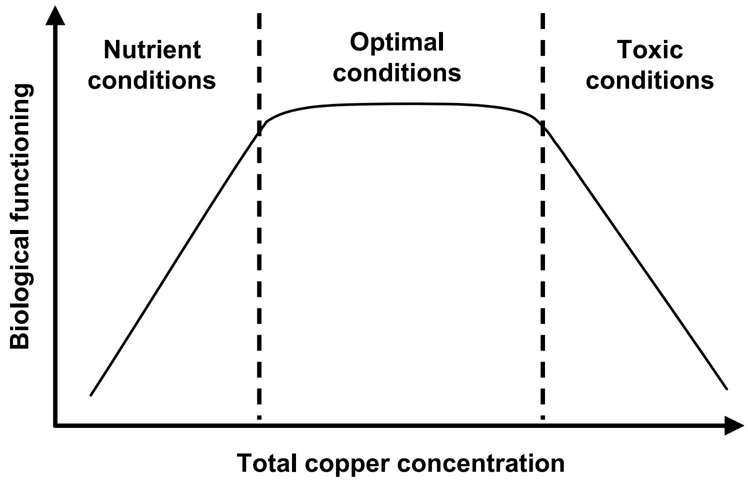


Figure 8. Schematic representation of the effects of total copper concentration on the biological health on a coastal area.

3.3 OVERVIEW OF METAL SOURCES IN COASTAL ZONES

The following discussion will focus on copper as an example. Copper is an abundant trace element present in all surface waters and sediments. Natural concentration ranges of dissolved copper in seawater are from 0.03 to 0.38 ppb (Chester, 1990) and from 0.2 to 30 ppb in freshwater. Anthropogenic activities can substantially impact these concentrations, with typical increases ranging from 0.5 to 5 ppb in harbors and estuaries, and higher levels in enclosed embayments and where there are large numbers of vessels or significant storm-water flows.

The presence of metals in aquatic environments has many sources. There are natural sources, including leaching from minerals with high metal concentration, atmospheric (i.e., eolian) transport, and deposition of metal-laden particles. There are a multitude of anthropogenic inputs of metals, which can be classified as point and non-point sources. This classification is mainly based in the physical mode of discharge to the aquatic environment. While point sources are delivered from specific, physically well-defined locations and therefore can potentially be controlled, non-point sources have physical dimensions that decrease or reduce the probability for control. Examples of point sources are industrial and municipal effluent discharges. Non-point sources include leaching from sediments at the bottom of harbors and estuaries; leaching from antifouling paints on the hulls of ships and boats; rain runoff from natural, urbanized, and industrialized areas; and eolian inputs. Storm

water is usually considered as a point discharge of diffuse sources (EPA, 1999). Some of these inputs are indicated for San Diego Bay in Figure 9.

Figure 9. Examples of point and non-point sources of copper in San Diego Bay, CA. Point sources are those with well-defined physically discharge, while those for non-point sources are not well physically defined. In this figure, non-point sources are runoff from urbanized/ industrialized from both civil and military areas.

3.4 METAL LOADING FROM NAVY SHORE SIDE OPERATIONS

A plethora of activities could result in metal discharges from Naval Facilities into the adjacent coastal waters. These include industrial activities, runoff and/or municipal land uses on naval installations. Most waste from municipal activities (i.e., housing, offices, commercial, industrial, etc.) is normally discharged into public-owned treatment works (POTW) or Navy-owned treatment works (NOTW), and can enter the coastal environment from these types of outfalls. Following is a discussion on the most prominent types of discharges that affect the levels of metals in the bodies of water where they are delivered. Copper sources are described as an example of these discharges.

3.4.1 Dry Docks in Shipyards

Copper is likely the primary metal of concern in the discharges from shipyards. Both copper and zinc are primary biocides in Navy antifouling coatings, and shipyard industrial activities can result in release of particles to the dry-dock floor and vicinity that include an antifouling coatings component. These activities include surface preparation, painting, metal plating, surface finishing, metal welding and cutting, machining and metal working, solvent cleaning and degreasing (Kura and Tadimalla, 1999). The excess copper concentration in the discharges from dry docks is a frequent problem, causing occasional regulatory exceedances and notices of violation (NOV; NAVFAC, 2003). Ship repair and recycling can also release copper. For example, Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) has at times experienced elevated levels of particulate copper from its ship dismantling and recycling program. The copper is picked up in rainwater or groundwater seepage and discharged through pumped point sources that are permitted under NPDES. Historically, copper discharges from the dry docks at PSNS have averaged 19 ppb or 0.4 to 0.8 pounds per day. To reduce copper loading, dry-dock process water was collected and treated with a system that initially cost $5.5 million (NAVFAC, 2003).

3.4.2 Storm-water Runoff

Storm-water runoff episodes are characterized by large metal concentrations in discharges from Navy shore-side operations. Runoff also transports particles containing copper (and other metals) from industrial areas where metal cutting and painting occur, from roadways and parking areas or from sources outside the facility. Copper sources also include leachate from copper piping and architectural uses, abrasion of vehicle break pads, and atmospheric deposition. Metal concentrations in storm-water runoff increase the regulatory pressure in a number of regions for capturing and treating storm water. Compliance related to the TMDL process will likely further impact capturing and treating storm water. The estimated cost to the Navy would be approximately $100 million to capture the first ¼-inch flush in the San Diego region (Brian Gordon, COMNAVREGION SOUTHWEST, personal communication.).

3.4.3 Copper Loading from Navy Fleet Operations

Normal operation of Navy vessels releases metals to the surrounding aquatic environment. These discharges are regulated under the amended Clean Water Act (Section 325 of the National Defense Authorization Act of 1996 (Pub. L. 104-106, 110 Stat. 254)) and covered under the Uniform National Discharge Standards (UNDS) joint U.S. EPA/DoD rulemaking process under authority of the provisions of the Clean Water Act (CWA Sections 312 and 502(6)). Once the rulemaking process for UNDS discharges is complete, ship discharges will not specifically come under TMDL/Waste Load Allocation regulation enforced by the states. However, the inclusion of discharges from ship operations may be considered in developing the total loading profiles for a region or watershed, and could affect the loadings from Navy shore-side operations. Ship discharges will be controlled through the joint rulemaking process by employment of marine pollution control devices (MPCDs), such as changes in antifouling coatings or fouling removal processes to significantly reduce or eliminate copper emissions from these paints.

The UNDS program has identified numerous copper and other pollutant sources associated with ship activities (U.S. EPA, 1998). Some of these contribute significant amounts of copper to the marine environment and are discussed below.

3.4.3.1 Leaching from Antifouling Coatings

Leaching of metals used as toxicants in antifouling coatings or in anodes is one of the most important inputs of copper and zinc to coastal embayments. This process occurs constantly, including when the vessel is underway and when it is docked. Since Navy vessels spend more time in port rather that underway, the passive release of copper when the vessels are docked is a major source of copper loading. Recent measurements of copper release rates directly off the hulls of Navy vessels, using an in-situ dome system, averaged 3.9 $\mu g/cm^2/day$ (Seligman *et al*., 2001; Valkirs *et al*., 2003), which is considered environmentally realistic for calculating total loading. Copper inputs to San Diego Bay were updated by Chadwick *et al*. (2004) to a good degree of certainty using this copper release measurement from Navy and civilian hull-coating leachates (Schiff and Diehl, 2002), civilian and Navy hull cleaning, other ship discharges (e.g., cooling water), point-source discharges, storm-water runoff, and atmospheric deposition (Johnson *et al*., 1998; PRC, 1997). Analysis of these data indicate total copper loadings of about 20,400 kg/yr and 22,000 kg/yr for dry weather and wet weather conditions, respectively, and that releases from antifouling paint are the main source

of copper, up to 65%, within the bay (Chadwick *et al*., 2004). The analysis also indicated that the distribution of copper sources in the bay is localized. The distribution of vessels seems to be the main factor affecting the distribution of copper sources in the bay (Figure 10). While the outer part of the bay (boxes 1 to 17) is dominated by pleasure boat sources, the inner part (boxes 18 to 27) is dominated by ship (i.e., commercial and military) sources.

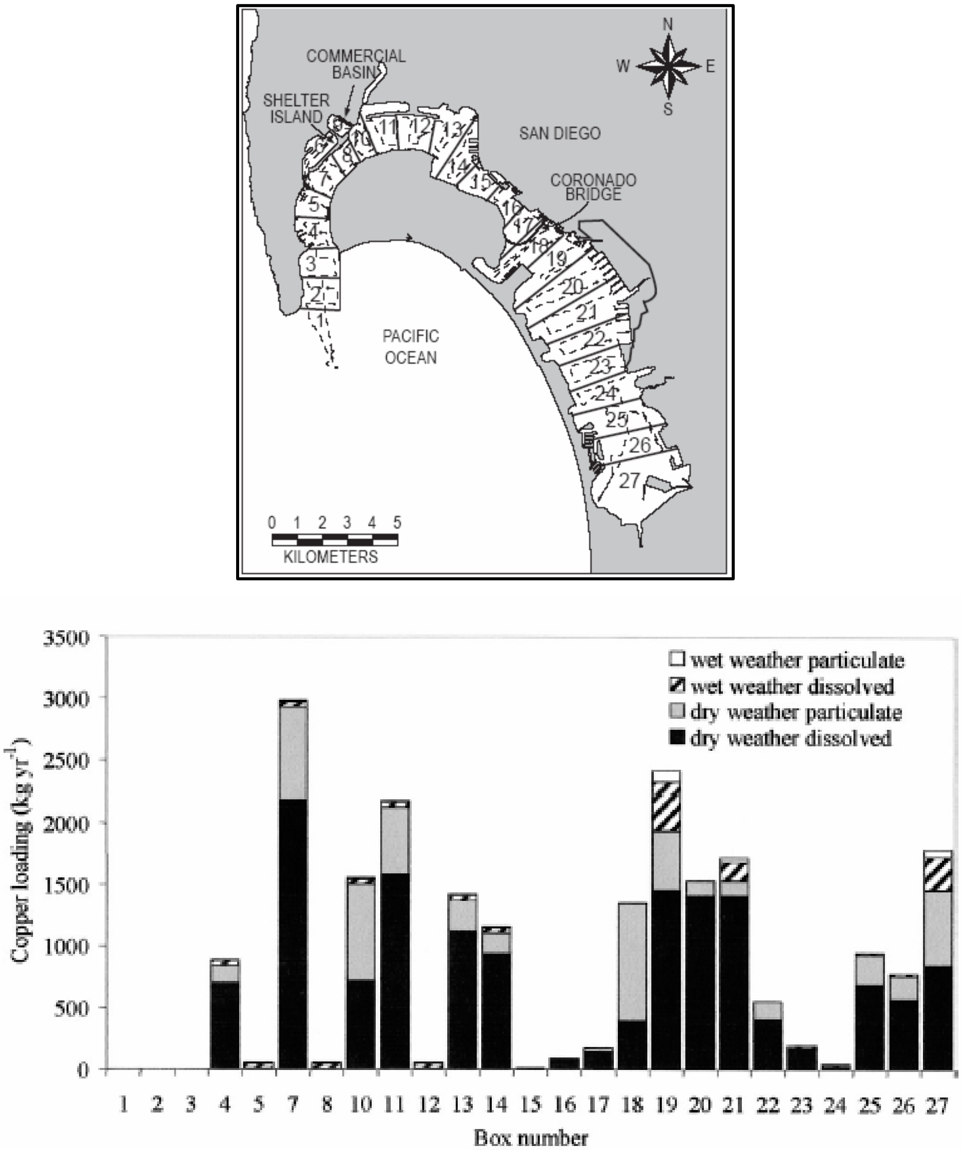


Figure 10. Estimated copper loading (kg/yr) in San Diego Bay (from Chadwick *et al*., 2004). The top figure shows the distribution of boxes assigned to San Diego Bay, and the figure at the bottom is the estimated copper loading at each box (Figures 1 and 2 in Chadwick *et al*., 2004).

3.4.3.2 Seawater Cooling

Contributions from cooling systems on Navy vessels are another important source of copper to the aquatic environment. The importance of this source is mainly due to the volume of water used, as opposed to the concentration of copper in the discharged water. According to NAVFAC (2003), the seawater discharged from cooling systems for all

armed forces vessels is estimated to be 390 × 10^9 gal/yr (390 billion gal/yr)., hence, it is important to have excellent accuracy in the measurement of metal concentrations in the discharged water, as it becomes a significant loading term. The cooling process is continuous, and since existing seawater cooling systems use copper in the heat exchanger, sea chest, pumps, and piping systems, copper is constantly dissolved, eroded, and released into the marine environment. The amount of copper in the effluent coming from the input water used in heat exchangers is considered similar to ambient levels in the source area (Earley *et al*., 2007).

3.4.3.3 In-Water Hull Cleaning

Pier-side underwater ship husbandry is a potentially significant contributor of metals to the environment. Measured concentrations of copper and estimated concentrations of zinc released from underwater hull scrubbing are significant and could exceed ambient water quality criteria (WQC). These high copper concentrations decrease rapidly, covering a relatively small spatial extent, as much of the copper is in particles and will settle to the bottom, adding to the sediment copper loading (Valkirs *et al*., 1994). Control of these discharges is accomplished by Navy policies in reducing the number of hull cleanings, using the least abrasive cleaning equipment, and limiting the cleaning to specific locations within a harbor.

3.4.3.4 Harbor Loading Assessments

The importance of the different inputs of copper to San Diego Bay is illustrated in Figure. The importance of antifouling coatings as sources of copper is also shown. It is useful to note that there are no official wastewater discharges to San Diego Bay, which could account for a substantial portion of the load in other coastal embayments. Another important difference compared with other harbors is that San Diego Bay, located in a desert area, is characterized by few rain events, with a minimal number of significant runoff events. Loading assessments have been performed for other harbors, including Pearl Harbor and Norfolk (Johnson *et al*., 1998); but these are not presented here. Although Figure 11 shows the distribution of inputs of copper in San Diego Bay, it does not show the specific source responsible for the metal content in a specific water sample. The example presented here is for a harbor that is critical to DoD, and a similar situation could be described for watersheds without a significant DoD presence, where copper inputs could originate from both municipal and industrial sources.

3.5 METAL FINGERPRINTING TECHNIQUES AND DATA ANALYSIS

The development and application of methods for tracking and fingerprinting metal sources in the aquatic environment is an active area of research. The overall goal of these efforts is to identify the sources of metals, information which can then be applied for subsequent targeted control of loading. Various demonstrated techniques can be applied, with the combination of considered the best approach to confirm sources and loading terms.

3.5.1 Concentration Gradients

A straightforward approach to source tracking is to use concentration gradients to pinpoint specific sources. This approach can be applied to any metal that is released in great quantities from a specific point source, as it only requires the ability to measure the concentration gradient superimposed on the distribution of natural concentrations. This was

the approach followed by Flegal *et al*. (1991) in San Francisco Bay for tracking copper sources. These authors identified two distinct biogeochemical regimes, one comprised by the estuarine mixing between the Sacramento/San Joaquin Rivers delta and the ocean in the northern section of the bay, and a regime perturbed by anthropogenic inputs in the South Bay. These regimes are discerned in plots of copper concentration versus salinity (Figure In an attempt to evaluate the contribution load from municipal waste water discharges to the estuary, Flegal *et al*. (1991) extrapolated the seawater copper concentrations in the South Bay to freshwater values following a simple dilution approach, finding projected copper concentrations in the wastewater discharges within a 95% confidence interval of the reported copper concentrations (Figure 12). These results demonstrate that the excess copper concentrations in the South Bay can be accounted for by inputs from wastewater discharges.

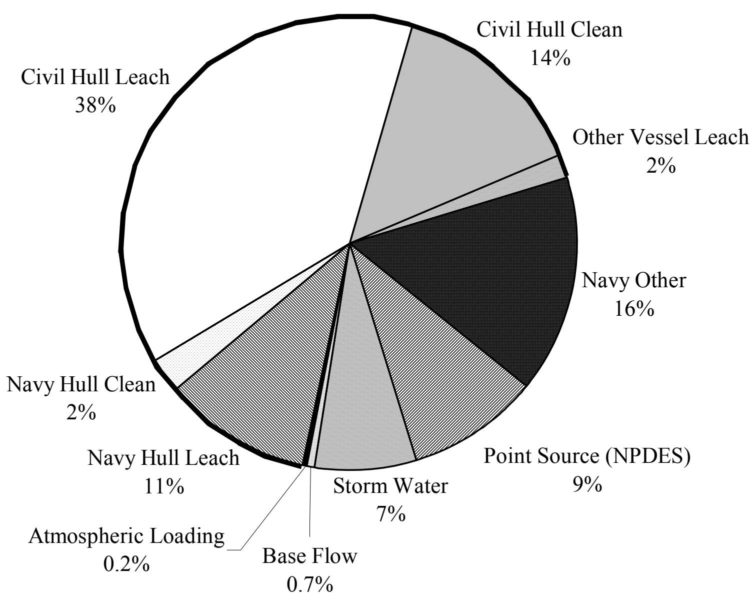


Figure 11. Distribution of inputs of copper to San Diego Bay (from Chadwick *et al*., 2005). The data was modified and updated from Johnson *et al*. (1998) and PRC (1997) to account for recent improvements in estimates for various input rates and to incorporate estimates for particulate copper (Chadwick *et al*., 2004). Those inputs that are related to antifouling paints are indicated by the bold outline, and are 65% to the total inputs to the bay.

3.5.2 Association to a Specific Source

Another tracking technique is to associate a metal to a specific natural or anthropogenic process. For example, cadmium concentration gradients have been related to upwelling processes and coastal water inputs into bays (Bruland *et al*., 1978; Bruland, 1980, Sañudo-Wilhelmy and Flegal, 1991), and silver has been associated to discharges from wastewater treatment facilities to coastal environments (Sañudo-Wilhelmy and Flegal 1992). The latter authors found a latitudinal variation in the ratio between lead and silver, indicating a two-component mixing model between San Diego's Point Loma sewage outfall (Pb/Ag ratio 3.2 to 3.3) and a background end-member (Pb/Ag ratio 5.3-6.2) representative of upwelling waters in the northeast Pacific (Figure 13). This information was used to estimate the contribution of wastewater effluents to those coastal waters in the Southern California Bight, which ranges from ≈ 94% at Imperial Beach, adjacent to the U.S./Mexico border, and to ≈ 19% at Punta Colonet, about 150 miles south of the border.

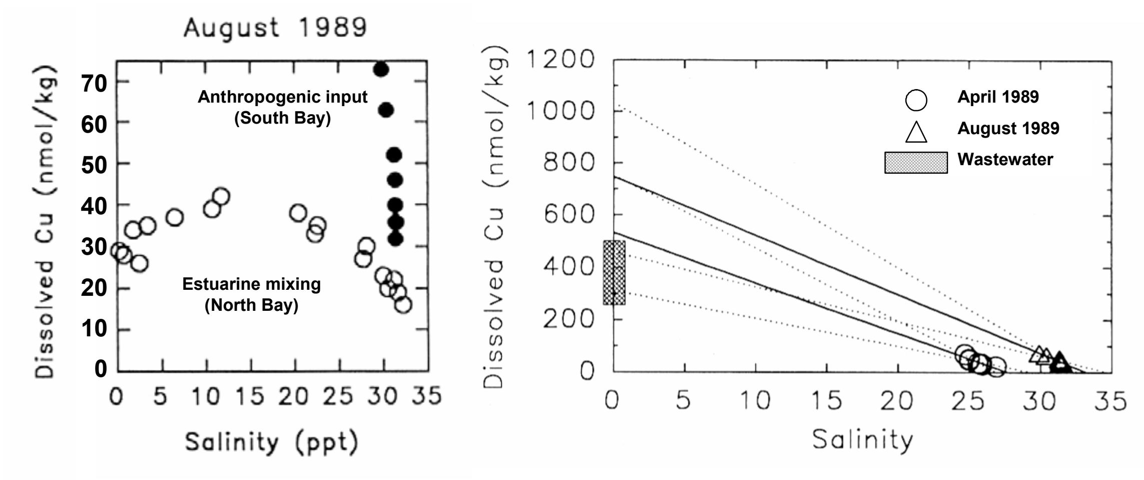


Figure 12. Example of the use of metal concentration gradients for assignation of metal loading to specific sources. The figure on the left is the dissolved copper versus salinity distribution in August 1989 in San Francisco Bay, where the regimes dominated by estuarine mixing and anthropogenic inputs are described. The figure on the right is the simple dilution (linear extrapolation) estimation of copper in wastewater sources in the South Bay (from Flegal *et al*., 1991).

Another similar approach is accomplished by assigning metals to specific industrial process. This is the case for the association of rare earth elements (REE) with petroleum-cracking catalysis. Olmez *et al*. (1991) compared the REE sediment composition in two sites, which showed enrichment in the light REE (lanthanum, cerium, neodymium, and samarium) within the top 36 cm of sediments collected in San Pedro Shelf, in comparison with the 60-cm core collected from Santa Barbara Basin. This enrichment of light REE in sediments was attributed to anthropogenic inputs that began in early 1960s from the Joint Water Pollution Control Plant wastewater located 6 km up current from the San Pedro Shelf sampling point. The sources of the light REE are petroleum-cracking catalysts and their products, including bottom ash, fly ash, and cracking catalysts, which are produced primarily from two REE minerals, bastnasite and monazite (Olmez *et al*., 1991).

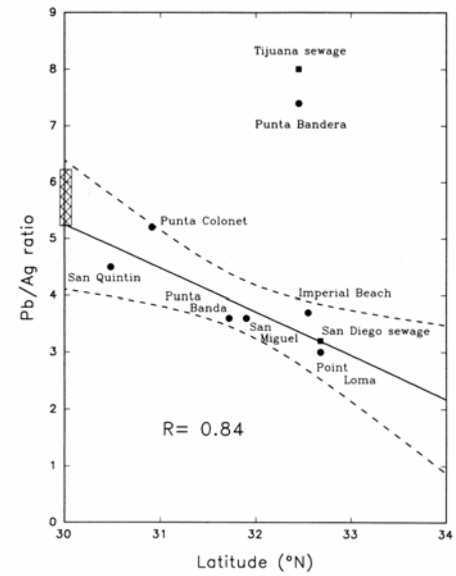


Figure 13. Association of lead/silver ratios to wastewater discharges. Latitudinal distribution of Pb/Ag ratios in coastal waters 2 to 5 km off shore in the Southern California Bight. The linear regression indicates a two end-member mixing model between upwelled water (cross-hatched grid) and wastewater effluents from Point Loma Wastewater Sewage Treatment Plant (Figure 3 in Sañudo-Wilhelmy and Flegal, 1992), with the 95% confidence limits in dashed lines.

3.5.3 Statistical Analysis

A more complex and commonly encountered scenario is a highly industrialized bay or estuary with multiple sources possessing the same general characteristics. There are several options for the assignment of responsibilities in this case. One option is using statistical analysis for the differentiation of sources. Statistical techniques such as cluster analysis and principal component analysis (PCA) can be used to classify information into groups with similar characteristics and to estimate the degree of responsibility for each discharger.

Montlucon and Sañudo-Wilhelmy (2001) used PCA to establish the control of groundwater and oceanic water on the chemical composition in waters from Flaunders Bay, USA (Figure 14). The authors, using PCA, discerned that there is an effect from both groundwater and oceanic water. However, a mass balance was required to understand the contribution from groundwater (10% and 58% in low- and high-aquifer recharge, respectively).

3.5.4 Fate and Transport Models

Another option for complex scenarios is using fate and transport models, which are algorithms developed for predicting the distribution of pollutants, given estimates of contaminant input loadings and knowledge of physical/hydrological forcing functions. The fate and transport models are also a tool for evaluating best management practices applied to point sources, or for the assignment of water quality targets, as in TMDLs. The quality of the information generated from these options is a function of the quantity and quality of the environmental data available.

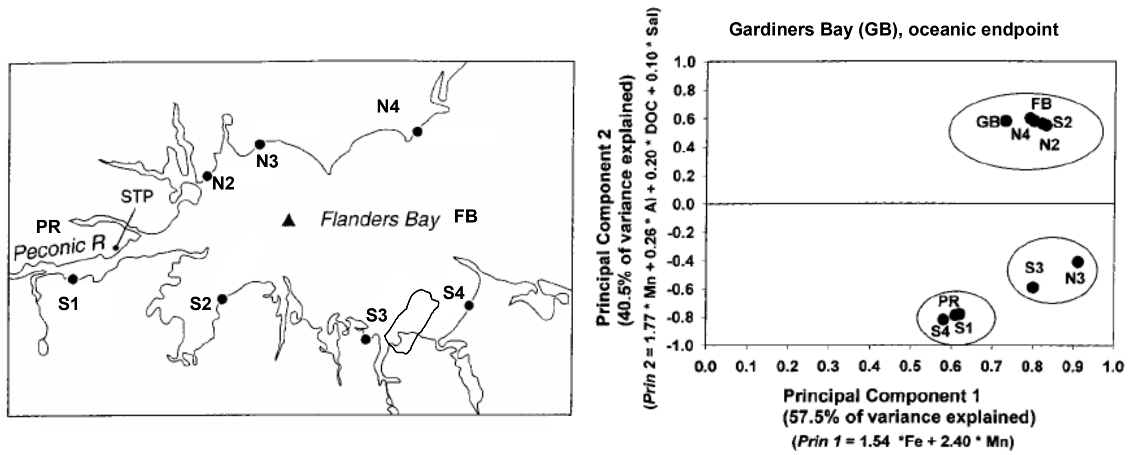


Figure 14. PCA analysis (left) in Flanders Bay, USA; Montlucon and Sañudo-Wilhelmy, 2001 Determined that both groundwater and oceanic water accounted for 98% of the variability of chemical composition.

In a study conducted in San Diego Bay, Wang *et al*. (2008) simulated the fate and transport of copper using the model TRIM2D. TRIM2D is a depth-averaged tidal and residual circulation model (Cheng *et al*., 1993) that has been calibrated and validated for inputs and concentration distributions of copper chemical species (i.e., total, dissolved and free copper) in San Diego Bay as part of Strategic Environmental Research and Development Program (SERDP) Project CP-1156, "Determining the Fate and Ecological Effects of Copper and Zinc Loading in Estuarine Environments: A Multi-Disciplinary Program" (Chadwick *et al*., 2005). Once TRIM2D was calibrated to simulate inputs of copper to the bay, it was possible to describe the current distribution of dissolved and free copper in San Diego Bay, and to evaluate hypothetical cases were either one of the two most important inputs are removed. These inputs include the leaching from antifouling coatings in Navy vessels, and from private vessels (Figure 15). This exercise emphasizes the importance of the fate and transport models as a tool for compliance and for optimization of resources in controlling inputs to coastal areas.

3.5.5 Isotopic Ratios

Source assignment in a complex situation could also be accomplished with isotopic ratios of stable isotopes. The basis of this technique is that the ratios of the stable (non-radiogenic) isotopes (i.e., isotopes that do not decay radioactively) of a given metal are fixed at the moment of the formation of the geologic ore from which the metal is extracted (Johnson, 2004; Zhu *et al*., 2002). Therefore, it is possible to differentiate and to estimate the relative contributions from several sources of the same metal by determination and comparison of the isotopic ratios in the original ores and in field/environmental samples.

The main assumptions associated with this approach are that the different ores or sources have a different ratio, and not affected by geological, biological, or environmental effects. The first assumption is required to differentiate the sources, and the second assumption assures that any measured isotopic ratio was generated from that source. Three mass-spectrometric techniques are used for highly precise isotopic ratio measurements. Isotope ratio mass spectrometry (IRMS) is used for stable isotopes of lighter elements (carbon, nitrogen, sulfur). Thermal ionization mass spectrometry (TIMS) is used for radiogenic (i.e., not stable) and some heavy stable isotopes (i.e., lead). The recently developed multiple-

collector inductively coupled plasma mass spectrometry (MC-ICPMS) allows for the analysis of most metals, including those inaccessible by the two other methods (i.e., copper and zinc; Felton, 2003).

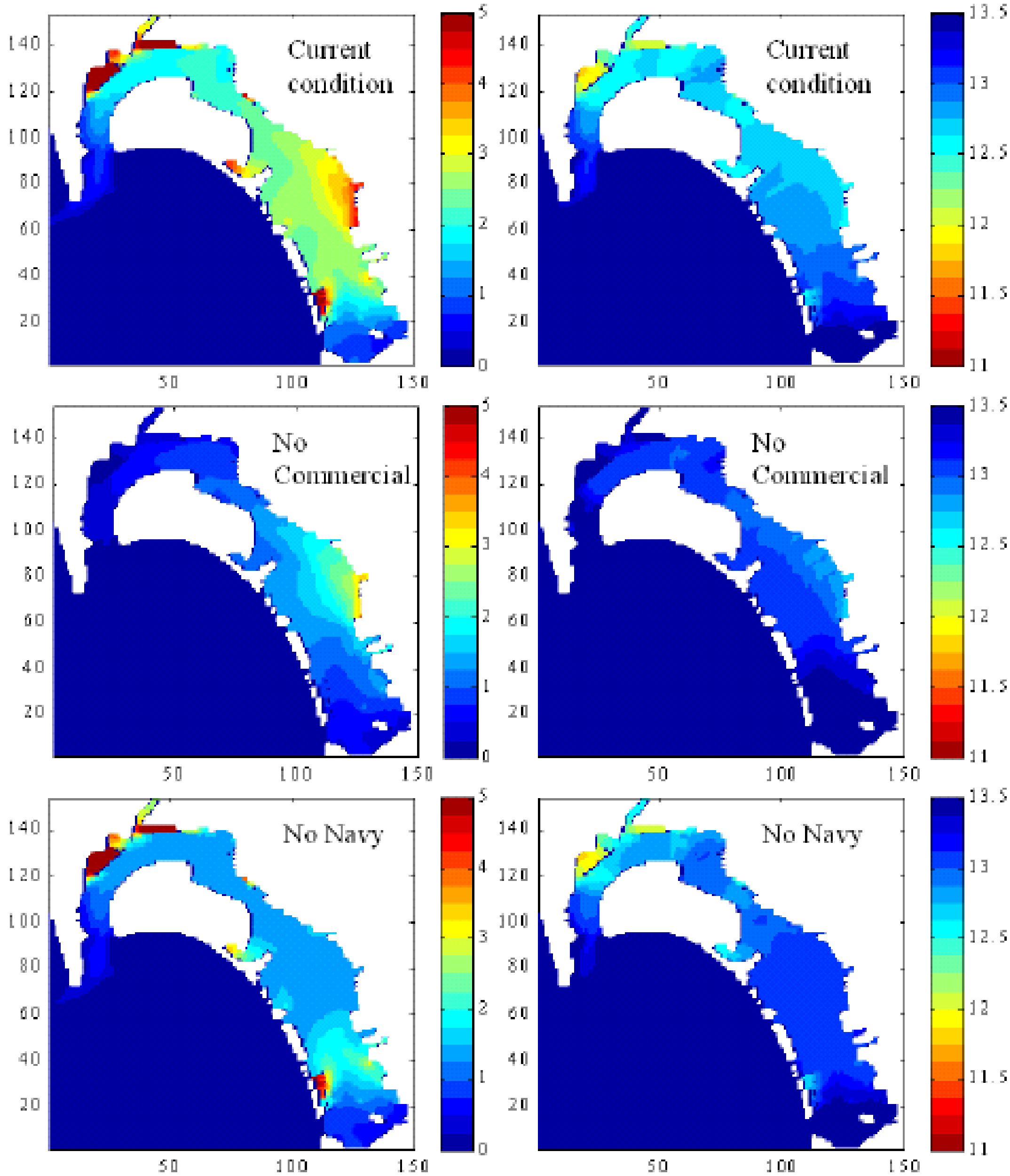


Figure 15. Predictions of dissolved copper (left panels, ppb) and free copper (right panels; -log [Cu^{2+}_{aq}] or pCu) distributions resulting from current and hypothetical copper loadings in San Diego Bay with the fate and transport model TRIM2D (from Wang *et al.*, 2008). The fate and transport model TRIM2D is used to predict the concentration distributions of these copper species in the cases were either one of the Navy or civilian (commercial) vessels antifouling coatings are removed.

Most isotopic ratio efforts for fingerprinting have focused on lead. This is because the analytical technique for the measurement of isotopic rations of heavier metals (TIMS) is more mature and has been more commonly available. A classical example using lead isotopic ratios is provided by the source differentiation lead between coastal waters and upwelled waters off the coast of California (Flegal *et al*., 1989). From a single vertical profile and satellite infrared images of sea surface temperature, this study identified the lead source in the upwelled water ($^{206}Pb/^{207}Pb = 1.17$) as Asian industrial lead ($^{206}Pb/^{207}Pb = 1.16$), while the surrounding coastal water ($^{206}Pb/^{207}Pb = 1.19$) is influenced by lead aerosols from United States ($^{206}Pb/^{207}Pb = 1.22$; Figure 16).

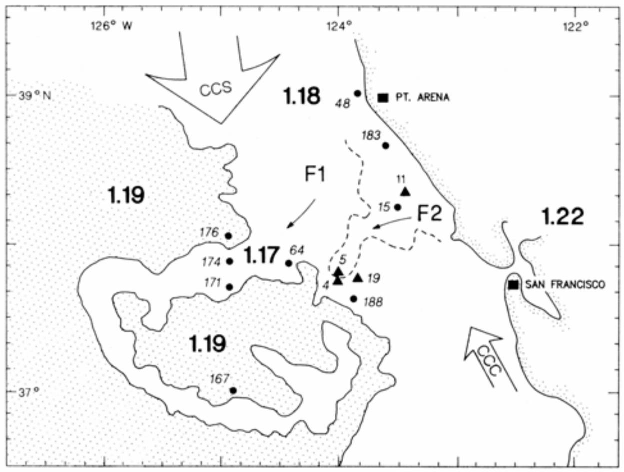


Figure 16. Use of lead isotopic ratios for identification of sources in coastal waters. Flegal *et al*. (1989) identified the source of lead in upwelled water ($^{206}Pb/^{207}Pb = 1.17$), as Asian industrial lead ($^{206}Pb/^{207}Pb = 1.16$), and the source of the surrounding coastal water ($^{206}Pb/^{207}Pb = 1.19$) as lead aerosols from United States ($^{206}Pb/^{207}Pb = 1.22$). The arrows indicate the flows of the California current system (CCS) and the countercurrent of California (CCC).

Recently, novel analytical techniques (MC-ICPMS) have been developed that enable the fingerprinting and tracking of copper using measured variations in ratios of the two stable copper isotopes (^{65}Cu and ^{63}Cu; Bermin *et al*., 2006; Zhu *et al*. 2000; Marechal *et al*., 1999). Subsequently, significant variation in copper stable isotope ratios in potential ore sources and environmental reservoirs were measured (Markl *et al*., 2006; Chapman *et al*., 2006; Marechal *et al*. 1999). In addition to these considerations, precise measurement of the small variation in isotope ratio is challenging. However, initial research on using copper isotopic ratios by Martin Shafer's group at the University of Wisconsin in Madison (Shafer *et al*., 2005), which included the measurement of copper isotopic ratios for several sources (Figure 17), has established critical, robust protocols for extraction, concentration, and clean-up of copper from environmental samples that were free of isotope fractionation artifacts. But application of copper isotopic ratios for tracking sources in aquatic environments has not yet been accomplished.

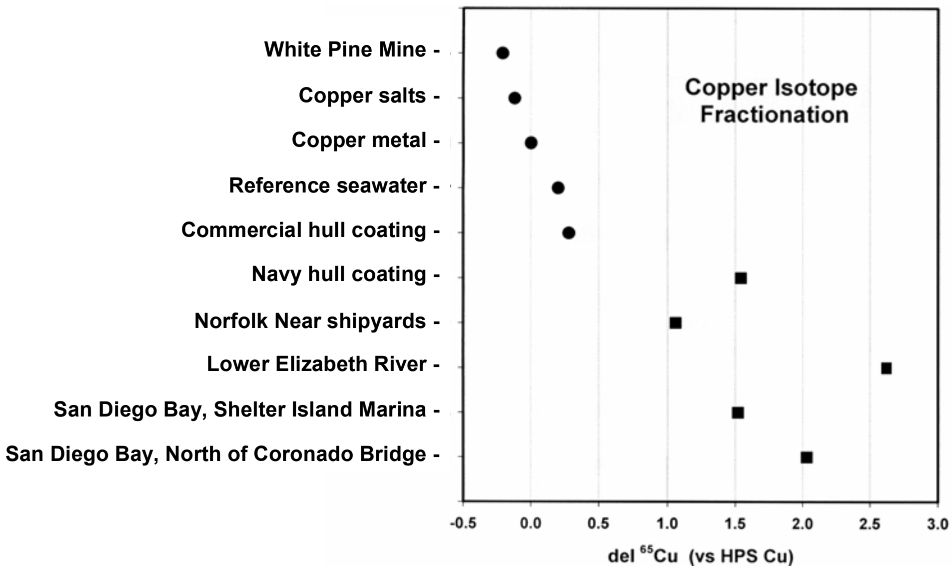


Figure 17. Copper isotopic ratios from a suite of sources, including antifouling coatings, and harbors heavily used by the DoD (Shafer *et al*., 2005).

3.6 ALTERATION PROCESSES

In general, metals do not undergo alteration or degradation processes. However, this statement refers to the total metal concentration in a body of water and must be explained as follows. In general, in a receiving body of water, the total concentration of a metal will be distributed between the water and sediments (i.e., reservoirs), and the total concentration of that metal in the body of water will only be affected by sources (inputs) and sinks (outputs) of the metal in the system. In contrast, within the reservoirs (water and sediment) the metal will be distributed to reach equilibrium. These considerations have minimal effect on tracking and fingerprinting metal sources, which generally provide metal concentrations in excess of those naturally present in most bodies of water.

For the tracking and fingerprinting techniques presented here, the primary alteration process to be considered is isotopic fractionation. This process is related to the natural processes that affect the ratios between the stable isotopes of a metal wherein a natural process might preferentially use a specific isotope. This effect has also been observed in lighter isotopes such as hydrogen, carbon, nitrogen, oxygen and sulfur. For example, plants prefer to take up carbon dioxide containing the lighter carbon isotope (^{12}C) rather than the heavier one (^{13}C) during photosynthesis; so for photosynthesis, the lighter carbon isotope (^{12}C) is enriched in organic matter. However, mass-dependent isotopic fractionation of metals during low-temperature environmental processing is generally quite small, and in the context of heavy metals (lead, copper), is considered to be a minor process contribution to the source fingerprint.

3.7 RAPID SCREENING TECHNOLOGIES

Rapid screening of the area of interest could provide significant information on the sources of metals. As rapid screening would provide variations of concentration over the area of study, and such an approach should be followed to define the source for the case where gradients in concentration exist. Only a few instruments are available for rapid screening. This document describes three: the Marine Environmental Survey Capability (MESC) at SPAWAR Systems Center Pacific (SSC Pacific), the ICP-MS system in a mobile clean-room of the Lawrence Livermore National Laboratory (Esser and Volpe, 2002), and the

Spectrophotometric Elemental Analysis System (SEAS) at the University of South Florida (Callahan *et al*., 2004). By no means, do any of these instruments cover the entire suite of rapid screening technologies; however, they are included here as examples of the types of instruments available.

3.7.1 Marine Environmental Survey Capability (MESC)

The MESC at SSC Pacific is a system designed for the characterization of spatial and temporal conditions in harbors and estuaries (Figure 18). It is a real-time data acquisition and processing system designed and built by the Navy to provide integrated, rapid, continuous measurement and synoptic mapping of oceanographic and environmental parameters (Chadwick and Salazar, 1991; Katz and Chadwick, 1993). The MESC system measures physical, chemical, and biological characteristics from the RV ECOS using state-of-the-art sensors, computer systems, and navigation equipment. This allows for direct, in situ measurements at a frequency commensurate with scales of natural and anthropogenic variability. The MESC real-time system employs both a towed sensor package and a seawater flow-through system that provides a continuous stream of seawater to a suite of onboard sensors.

Included onboard the MESC is a Trace Metal Analyzer (TMA), which is an instrument designed for the continuous measurement of metal concentrations in near real time. The system (Figure 19) consists of a computer, custom control, data acquisition and analysis software, a custom computer-controlled potentiostat with data acquisition circuitry, and a custom flow-through electrochemical cell module with sample handling components. The TMA was designed for automated collection and analysis of ppb levels of heavy metals in water using Potentiometric Stripping Analysis (PSA). PSA is performed by applying a voltage potential to the working electrode (glassy carbon rod tipped by a thin gold disk) to deposit metal from the water onto the electrode (reduction of metal forming an amalgam on the electrode), and then the voltage is removed and the potential of the electrode is measured. The potential drops until it reaches a characteristic value for that metal oxidation state. At this voltage, the metal is oxidized and “stripped” off of the electrode. The potential remains constant until all of the metal is oxidized, producing a plateau in the voltage versus time graph. The width of the plateau is proportional to the concentration of the specific heavy metal originally in solution. Normally, the TMA uses the method of standard additions, with an analysis time of approximately five minutes per sample.

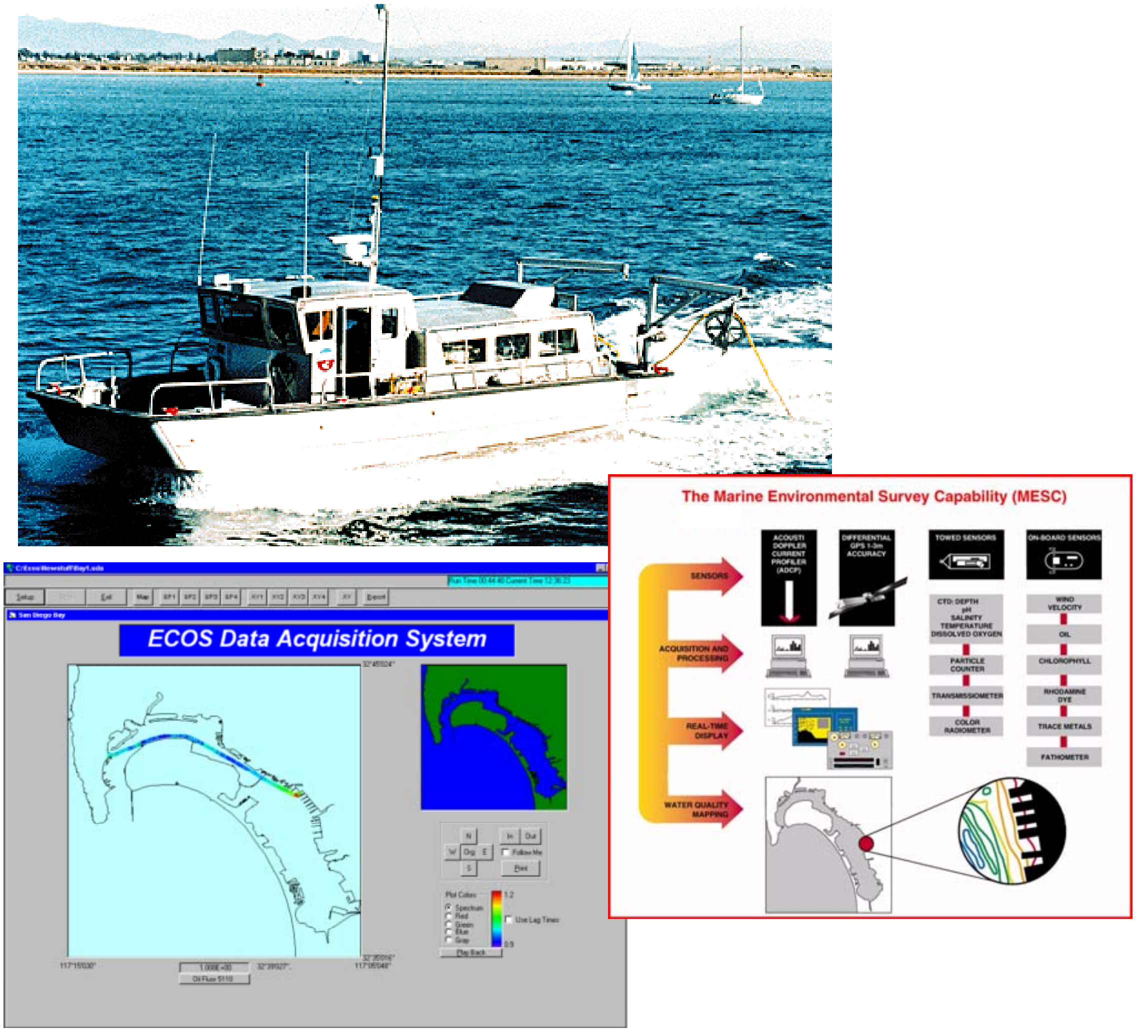


Figure 18. SSC Pacific's Marine Environmental Survey Capability (MESC) instrument. The MESC is an automated real-time system for the characterization of spatial and temporal conditions in harbors and estuaries.

3.7.2 At-Sea Inductively Coupled Plasma Mass Spectrometer

Bradley Esser and Alan Volpe, from the Laurence Livermore National Laboratory (LLNL), modified an ICP-MS for use at sea (Esser and Volpe, 2002). They accomplished this by incorporating shock-absorbing structures to isolate the ICP-MS from high-frequency shipboard vibration, and by housing the instrument in a mobile clean room. Clean rooms are enclosures where the amount of particles in the air is controlled by filtration through high efficiency particulate air (HEPA) filters, resulting in a positive air pressure. These conditions ensure the minimization of metal contamination from particles in the air. The system also includes an online preconcentration step, and seawater is filtered through 0.42-µm pore-size filters before analysis, effectively constraining the measurements to dissolved metal concentration. Limits of detection with this instrument are in the range of 0.003 ppb for copper and nickel, and 0.03 ppb for zinc (Esser and Volpe, 2004).

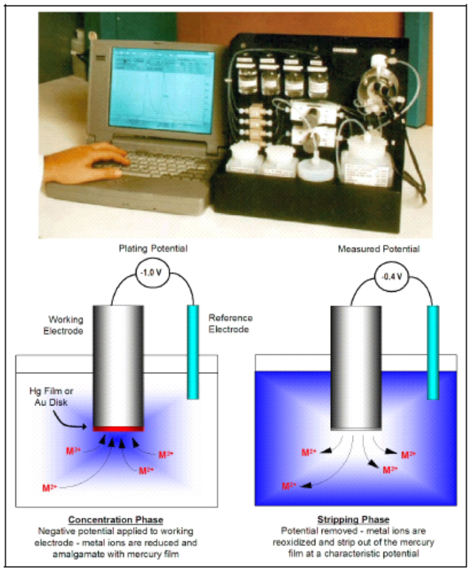


Figure 19. The Trace Metal Analyzer (TMA) of SSC Pacific, and a conceptualization of the potentiometric stripping analysis. The TMA measures metal concentrations in near real time.

3.7.3 Spectrophotometric Elemental Analysis System (SEAS)

The Callahan group Spectrophotometric Elemental Analysis System (SEAS) was designed for real-time in-situ measurements of trace metals and nutrients in aqueous solutions (Callahan *et al.*, 2004). This instrument measures the absorption of light through a novel optical cell, which is then used to calculate the concentration. The sensitivity depends on the optical path length, and by using a liquid core waveguide (a flexible tube made from Teflon AF-2400), the SEAS achieves optical path lengths of up to 10 meters. This path length yields measurement resolutions in the low parts per trillion (pptr) range. Added benefits of this design are the small sample volume (0.5 ml, typical), and small-instrument size that is achieved by using a coiled waveguide (Figure 20).

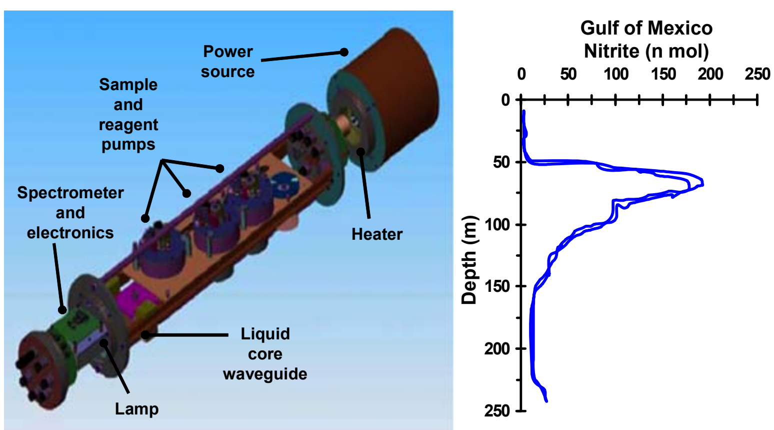


Figure 20. The Spectrophotometric Elemental Analysis System (SEAS) from the University of South Florida. The diagram on the left shows the components of the SEAS and on the right is a depth profile for nitrites measured in the Gulf of Mexico with the SEAS. From: http://www.marine.usf.edu/PDFs-and-DOCs/publications/R.Easley-ASLO2006.pdf.

3.8 ANALYTICAL CONSIDERATIONS

3.8.1 Trace Metal Clean Sampling and Analysis Techniques

One of the greatest difficulties in tracking sources of metal contamination is successfully precluding sample integrity during collection, transport, and analysis. Trace metal concentrations in the aquatic environment are typically in the low-ppb or sub-ppb concentration range (e.g., see Chester, 1990, page 347, for metal concentrations in seawater), and artificial increases in metal concentration (i.e., sample contamination from the natural environment) during handling of samples could introduce bias into the identification and quantitation of excess metal concentration, and the potential to track the source(s) of the metal. The USEPA is aware of these sample-handling issues and has released guidance for Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels (US EPA, 1996). These techniques have been routinely used in the environmental research community and are known as trace metal ultra clean-techniques. The methodology includes using metal-free sampling containers and analytical equipment, and working in spaces with high efficiency particulate air (HEPA) filters. The goal for using these techniques is to avoid incidental contact with and introduction of environmental particles into the sample, with HEPA filtration eliminating particles of approximately 0.2 µm and larger in diameter. HEPA filtered working areas are classified by the number of particles per cubic feet of air, thus a class-100 working area is recommended for trace metal analysis of aquatic environmental samples.

3.8.2 Analytical Techniques

Another important consideration in tracking metals in the aquatic environment is the sensitivity (i.e., limit of detection) of the analytical technique of choice. Indeed, a comparison of the Water Quality Criteria (WQC) and the analytical capacity can be made. In the case of copper, initial WQC has been typically based on total copper concentration, as this is the main chemical fraction possible to measure. Improvements in sampling and analytical techniques have made possible the measurement and use of dissolved copper, which in seawater is a fraction of the total copper concentration. As a result of this improvement, the regulatory effort was modified to focus on the dissolved fraction (US EPA, 1993). Recently, the US EPA released a draft WQC for copper in freshwater systems (U.S. EPA, 2003) based on the Biotic Ligand Model (BLM; Di Toro *et al*., 2001; Santore *et al*., 2001). This model estimates the amount of copper in the freshwater that will have an expected toxicity end-point. This estimation takes in consideration the chemical fractionation in the freshwater, including the free copper ion concentration. Therefore, the proposed WQC for freshwater is based in the free copper ion, a fraction of the total copper, for which the recent development of analytical techniques supports.

A list of analytical techniques is provided in Table 1 (U.S. EPA 1996, Table 3-1), which are suitable for the analysis of aquatic environmental samples (collected following trace metal clean procedures). This table shows that the metalloids mercury (Hg) and arsenic (As) must be measured with ion specific analytical techniques. The specified method for measurement of hexavalent chromium (Cr^{6+}, or Cr(VI)) is ion chromatography. The list also indicates that most metals can be measured by either stabilized temperature graphite furnace atomic absorption (STGFAA) spectrometry or by inductively coupled plasma with detection by mass spectrometry (ICP-MS). Also included in the table is the use of techniques for preconcentration of the heavy metal (this is indicated in Table 1 as CC, the acronym for Chelex® column). This type of column is a resin with high affinity for heavy metals and low

affinity for sodium, allowing the preconcentration of the metal with concurrent reduction in the salt concentration (e.g., sodium chloride). Other preconcentration techniques commonly used for measurement of heavy metals in seawater at sub-ppb levels, including, for example, liquid-liquid preconcentration with dithiocarbamates (Bruland *et al*., 1985), co-precipitation with cobalt and pyrrolidinedithiocarbamate (Bloom and Crecelius, 1984), and co-precipitation with $Mg(OH)_2$ (Weiss et al., 2000), and use of 8-hydroxyquinoline as chelating resin (Orians and Boyle, 1993). A current list of USEPA- approved Clean Water Act methods can be find at the USEPA website: http://www.epa.gov/waterscience/methods/method/index.html. A summary of techniques, detection limits, advantages, limitations, unit costs, and maturity levels is shown in Table 2 and detailed in the following sections.

3.8.2.1 Stabilized Temperature Graphite Furnace Atomic Absorption (US EPA Methods 200.9, 200.12, 200.13)

This technique is probably the least expensive technique available for measuring metals in a natural concentration range. It delivers a precise volume of sample onto a graphite platform located inside a graphite tube, and then applies a thermal treatment to volatilize atoms of the metal of interest in the ground state into the inert atmosphere inside the tube (Figure 21). These atoms absorb energy at wavelengths specific for each metal, and the absorption is proportional to the concentration of metal in the sample. A source lamp at the metal-specific wavelength (i.e., the lamp in Figure 21) and a photodetector are required.

Advantages of STGFAA. The precision and detection limit of the STGFAA are the main advantages. Development of high precision sample delivery instruments, highly stable lamps and detectors allows for excellent precision and low-detection limits. The technique is simple, and on average it takes a few days to train a new user to use the instrument.

Limitations of STGFAA. The primary disadvantages of STGFAA include interferences by the salt content of the sample, time of analysis per sample per metal, limit of detection. The thermal treatment results in deposition of salts in and around the graphite furnace itself. This salt will decrease the number of samples that can be run, especially when analyzing seawater. One way to eliminate or control this problem is by preconcentration of the sample, as this increases the amount of metal available for detection and decreases the amount of salts present in the sample. Preconcentration also improves the limit of detection of GFAA to sub-ppb levels for most heavy metals. Time of analysis is extensive, as most STGFAA instruments are designed for the analysis of one heavy metal per run. A typical run of about 40 samples takes an average of 8 to 9 hours to be completed.

Cost of Metal Analysis by STGFAA. As previously mentioned, of the analytical techniques available for quantitation of heavy metals at the low ppb and sub-ppb level, STGFAA would be least expensive. Instruments for STGFAA are on the order of \$50 to \$75K, in contrast to an ICP-MS, which will cost about \$275K. However, the most common scenario would be for the DoD user to contract a private analytical laboratory for quantitation of the metal. Costs for this approach are on the order of \$25 per sample per metal.

3.8.2.2 Inductively Coupled Plasma Atomic (Optical) Emission Spectrometry (US EPA Methods 200.7)

One might want to evaluate the concentration of metals present at relatively high concentrations in some instances. This includes metals in sediments, and metals that are naturally present in high concentrations in aquatic environments such as sodium in seawater,

and aluminum, iron and manganese in suspended particles. For these applications, using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), also known as ICP-Optical Emission Spectrometry (ICP-OES), is advised.

Table 1. This table is Table 1 from USEPA (1996), and indicates analytical techniques available for evaluation of metal concentration in aquatic samples. CC indicates use of preconcentration by Chelex® Column, STGFAA is stabilized temperature graphite furnace atomic absorption spectrometry, ICP is inductively coupled plasma, and MS is mass spectrometry.

TABLE 1. ANALYTICAL METHODS, METALS, AND CONCENTRATION LEVELS APPLICABLE TO METHOD 1669

Method	Technique	Metal	MDL (µg/L) [1]	ML (µg/L) [2]
1631	Oxidation/Purge & Trap/CVAFS	Mercury	0.0002	0.0005
1632	Hydride AA	Arsenic	0.003	0.01
1636	Ion Chromatography	Hexavalent Chromium	0.23	0.5
1637	CC/STGFAA	Cadmium	0.0075	0.02
		Lead	0.036	0.1
1638	ICP/MS	Antimony	0.0097	0.02
		Cadmium	0.013	0.1
		Copper	0.087	0.2
		Lead	0.015	0.05
		Nickel	0.33	1
		Selenium	0.45	1
		Silver	0.029	0.1
		Thallium	0.0079	0.02
		Zinc	0.14	0.5
1639	STGFAA	Antimony	1.9	5
		Cadmium	0.023	0.05
		Trivalent Chromium	0.10	0.2
		Nickel	0.65	2
		Selenium	0.83	2
		Zinc	0.14	0.5
1640	CC/ICP/MS	Cadmium	0.0024	0.01
		Copper	0.024	0.1
		Lead	0.0081	0.02
		Nickel	0.029	0.1

[1] Method Detection Limit as determined by 40 *CFR* Part 136, Appendix B.

[2] Minimum Level (ML) calculated by multiplying laboratory-determined MDL by 3.18 and rounding result to nearest multiple of 1, 2, 5, 10, 20, 50, etc., in accordance with procedures used by EAD and described in the EPA *Draft National Guidance for the Permitting, Monitoring, and Enforcement of Water Quality-Based Effluent Limitations Set Below Analytical Detection/Quantitation Levels*, March 22, 1994.

Table 2. Summary of metal analytical techniques.

Technique	Detection Limit	Advantages	Limitations	Unit Cost (K)	Mature?
Flame-AA	high ppb	Single metal	Many interferences Not automatic	$35K	Yes
STGFAA	sub ppb	Single metal Automatic	Salt interferences	$60K	Yes
CC/STGFAA	sub ppb	Single metal	Extra sample handling	$60K	Yes
ICP-AES	sub ppb	Multiple metals	Salt interferences	$100K	Yes
ICP-MS	sub ppt	Multiple metals	Salt interferences	$275K	Yes
MC/ICP-MS	sub ppt	Multiple metals Isotopes possible	Extra sample handling	$750K	Yes

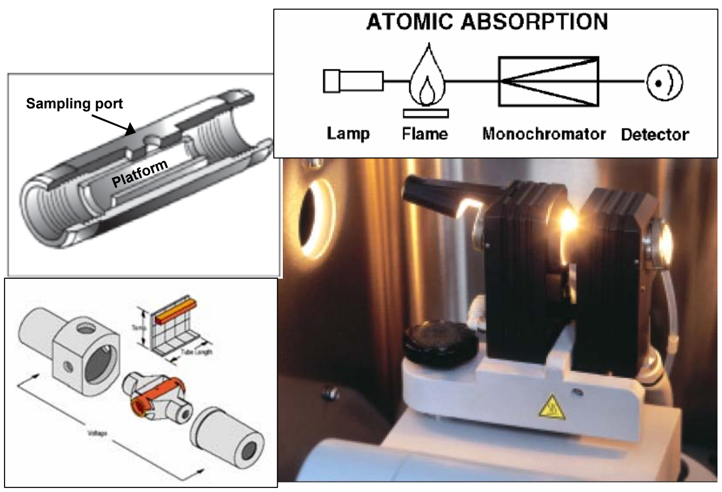


Figure 21. Diagram of two types of graphite tubes with platform and a STGFAA at the atomization step. The diagrams and the picture are from the Perkin-Elmer website: http://las.perkinelmer.com/Catalog/default.htm?CategoryID=Atomic+Absorption+%5bAA%5d)Advantages of STGFAA.

In ICP-AES (or ICP-OES), a spectrometer measures the amount of radiation emitted from the sample. The radiation from the plasma is focused into the spectrometer, where a monochromator is used to separate the individual wavelengths onto the detector. In older instruments, the detector usually is composed of a series of photomultiplier tubes (PMTs), whereas newer instruments use solid-state detectors that detect all wavelengths at one time without using a monochromator. The concentrations of the heavy metals of interest are determined by comparison to known-concentration standards, or by the method of standard addition.

Advantages of ICP-AES (ICP-OES). The main advantage of ICP-AES (or ICP-OES) is the large number of heavy metals that are analyzed for each injection of sample. Typical instruments can analyze up to 25 elements in 5 minutes per sample. However, the limit of detection of ICP-AES (or ICP-OES) is at least an order of magnitude larger than for STGFAA. Examples of detection limits and a description of the system are provided by Perkin-Elmer in the Guide for Inorganic Analysis at the website: http://las.perkinelmer.com/content/Manuals/GDE_InorganicAnalysis.pdf. These detection limits (summarized in Table 2) support the use of ICP-AES (ICP-OES) for heavy metal determination in soils, sediments, wastewater and other matrices with relatively high concentrations of metals.

Limitations of ICP-AES (ICP-OES). The primary limitation of this technique is likely the relatively large limit of detection (i.e., tens of ppbs). The ICP-AES (or ICP-OES) is more efficient in waters with low salinity (a salinity of 3 or less), thus a preconcentration step is required for seawater to bring the metal concentration in the sample to measurable levels. In addition, a digestion step is also required for the analysis of soils and sediments.

Cost of Metal Analysis by ICP-AES (ICP-OES). The price of instrumentation is on the order of $100K. However, as indicated above, for a DoD user commonly contracting services to a private laboratory, the price per analysis is typically in the range of $10 per sample per element.

1. 3.8.2.3 Inductively Coupled Plasma Mass Spectrometry (U.S. EPA Methods 1640, 200.8)

Inductively coupled plasma mass spectrometry (ICP-MS) is arguably the best analytical instrument available for measuring heavy metal concentrations at environmental levels. This instrument can measure heavy metal concentrations at the sub-part-per-trillion detection limits, has multi-element capabilities, and is able to provide isotopic concentrations (Cottingham, 2004). The analytical design of this instrument is similar to the ICP-AES, with respect to the use of Argon plasma, with the primary difference being in the detection method. ICP-MS uses a mass spectrometer for the detection of the isotopes of interest.

Advantages of ICP-MS. As indicated above, these instruments can quantify, simultaneously, several heavy metals at sub-part-per-billion concentrations, and can quantify the isotopic composition of the sample.

Limitations of ICP-MS. Similar to STGFAA and ICP-AES (or ICP-OES), ICP-MS is affected by the presence of dissolved and suspended solids. Again, ICP-MS works more efficiently with freshwater or very low salinity samples and preconcentration is required for analysis of seawater. Online preconcentration is available at private analytical laboratories. An added limitation is the increased level of preparation required for operation of the instrument.

Cost of metal analysis by ICP-MS. The cost of an ICP-MS system range between $75 to $750K or more, depending on the sophistication required. An ICP-MS in the lower range should be able to measure concentrations at regulatory levels. Contracting for on-line preconcentration ICP-MS is expensive, with charges on the order of about $250 per sample for one metal, including preconcentration, plus an estimated extra $70 for each metal per sample.

3.9 TECHNOLOGY GAPS

Tracking and fingerprinting metals in the aquatic environment needs a great deal of detailed planning. From the examples presented here, note that each scenario requires a different set of parameters and a different approach for the assignation of source. In addition, as this is a current topic for basic and advanced research, new approaches are constantly being developed.

Probably some of the most important gaps identified in the available technology relate to applying isotopic ratios for copper fingerprinting. These include three main components: (1) validation of this approach, (2) information regarding the characterization of fractionation of copper isotopes in nature, and (3) common availability of instrumentation for measurement of copper isotopic ratios. Dr. Shafer's group at UWM has pioneered the application of copper isotopic ratios for fingerprinting; however, the validation of this approach has not been completed, with sampling and analysis of other source end-members and analysis of many previously collected samples still outstanding.

As indicated above, differential fractionation of the isotopes is a potential problem. While the information available appears to indicate that there is no fractionation for copper, this issue needs confirmation,, i.e. via basic research on changes in copper isotopes ratios by different natural processes.

As also indicated above, only one type of instrument can measure copper isotopic ratios—the MC-ICPMS. This instrument is expensive (≈$750K to $1M), and is not available yet in commercial laboratories. Therefore, to use copper isotopic ratios, the user must contract the isotopic ratio analytics to academic entities, which could require additional funding or time for the effort.

3.10 CONCLUSIONS

As described above, a suite of approaches is available for the tracking and fingerprinting of metals in aquatic environments. These approaches address continued regulatory efforts to control the effect of metal releases in aquatic environments, which include compliance and cleanup activities. To effectively target control and remediation strategies, reconciling the various contributing sources of contaminants is critical. These sources are both of natural and anthropogenic (synthetic or man-made) origin. Tracking and fingerprinting approaches for the identification of the original source of metals, or the loads attributable to different sources into aquatic environments include: (1) concentration gradients, (2) association of the metal with a specific source, (3) differentiation of sources using statistical analysis, (4) application of fate and transport models for the elucidation of sources and effects, and (5) fingerprinting the sources with isotopic ratios. The successful application of any of these approaches is determined by the characteristics of the area of study. Some of these approaches are less mature than others, and further development is required in order to substantiate their application.

3. MICROBIAL SOURCE TRACKING

4.1 INTRODUCTION

Waters impacted with fecal contamination can affect human and animal health due to effects of disease-carrying pathogens and viruses. Fecal contamination can cause financial losses due to shut-downs of recreational activities or food harvesting associated with the water (drinking, swimming, or seafood harvesting).

Identifying sources of bacterial pollution are often not clear cut but are necessary prior to resolve compliance issues and target complete remedial actions. Certain bacteria and viruses serve as indicators for a wide range of pathogens, and these organisms are those targeted for microbial forensics. (EPA, 2002b)

Microbial forensics is also often referred to as Microbial Source Tracking (MST) or bacterial source tracking (BST) and has also been called Pathogen Source Tracking (EPA, 1994). In 1994, the US EPA had guidelines that if the bacterial contamination in waterways was due to an animal input, then standards could be waived as it was believed that there was no correlation between swimmer illness and animal pathogens. However, this has been revised as data since then do indicate that non-human feces do present potential health risk to swimmers and the waiver has been eliminated (EPA, 2002a).

4.2 NAVY BACTERIAL SOURCES

The Navy has had some concerns with bacteria TMDLs at some of their sites. Below is a breakdown for Navy TMDL listings for bacteria from the Naval Facilities Engineering Command Prioritization Report (NAVFAC, 2003). The most likely use of microbial source tracking by Navy managers involved in bacterial TMDL calculations will be during TMDL implementation in the identification of locating and remedying sources of fecal bacteria.

Navy facilities (particularly industrial facilities) are a potential source of fecal impairment and may be impacted by fecal TMDLs. When it is known that the fecal impairment is due to human activities, there are inexpensive methods that can be used to determine the source of the fecal contamination. When the cause and/or the source of the impairment is unknown, there are MST methods that can be utilized to determine bacterial sources. Some of the following are reasons for potential Navy facilities to have fecal impairment:

- Navy facilities are generally located on the water in urban areas
- Fecal impairment is common in urban, near-shore areas
- Most Navy facilities are old, some were built during a time when combined storm/sanitary systems were common; or when industrial discharges were not treated
- Large population of workforce
- Facilities have been upgraded to separate the sanitary systems, but designs of the sanitary systems do not necessarily meet current design practices; for example, the new sanitary lines are commonly run in combination with existing storm-water pipes
- Although sanitary systems may have been separated from the storm-water systems, many of the pipes are old and have exceeded their design life
- The maintenance of in-ground utilities has not always been adequate—repair dollars have gone to more visible improvements

- Problems with the sanitary systems can go undetected
- Facilities are often built on fill material increasing the potential for pipe breaks due to settling
- Repairing storm and sanitary lines can be extremely expensive due to the high density of in-ground utilities
- Navy facilities have been concentrating on other pollutants (metals, PCBs, mercury, etc.) and have not considered the potential for fecal problems

4.3 BACKGROUND

Total maximum daily loads (TMDLs) are regulatory mechanisms for establishing an excess of contaminant loading that needs to be addressed due to a calculation of the maximum amount of a pollutant that a water body can receive and still meet water quality standards. When discussing bacterial TMDLs, this is generally a reference to pathogenic bacteria; and pathogens could refer to bacteria, protozoans, and viruses associated with human or other warm-blooded animal fecal waste (EPA, 2002a). When water quality standards (WQS) are exceeded, this can result in beach closings, which may have an effect on economic development, agricultural activities, and the health of a population. However, TMDLs also affect the Navy as most Navy installations are near water. In California, Florida, Hawaii, Virginia, and Washington, the Navy has a large presence, and approximately 240 TMDLs approved since 1996 and more than 3400 impaired waters were identified. Of these impairments, pathogens were listed second only to sediment/ siltation (Kathy Ellis, 2002). This chapter/section will focus on bacterial TMDLs and mechanisms to determine potential contaminant sources.

A simple way to track pollution is to identify and track indicator organisms (indicative of fecal contamination) (Ioana G.Petrisor et al., 2006). Generally, MST and BST primarily refer to the tracking of fecal contamination and, as of this review, are not used to represent any other bacterial contamination. The primary reason to perform MST is to establish an accurate assessment of the source of fecal pollution in waterways such as ponds, streams, estuaries, rivers, and oceans. The principal concern is on human health, but occasionally, environmental health. Once an accurate source of contamination is established, we can then address effective remediation and prevention.

Bacterial and viral pollution can come from anywhere, but in this particular area, we are more concerned with pathogenic organisms. For TMDLs, pathogenic organisms in water sources are generally from fecal matter from animals or humans. Testing for all potential pathogens is difficult; therefore, environmental scientists usually only test for total or fecal coliforms. Coliform bacteria are a group of gram negative bacteria that are aerobic and lactose fermenting (can use lactose sugars); Escheria coli (*E. coli*) members belong to the coliform bacteria. However, not all coliform bacteria pose a health threat to humans; only a minority of *E. coli* strains pose a health threat. Many coliform bacteria are found in the gut tract of humans and animals; some are common in soils and sediments and some are associated with plants. Identifying these organisms in diagnostic libraries (incubated at 35°C on specific media) is usually simpler, which is one reason we use them to detect the potential presence of fecal pathogens. Fecal coliform bacteria are coliforms usually only found in the gut tract of humans and animals. Again, not all fecal coliform bacteria are pathogenic (i.e., Escherichia, Enterobacter, Klebsiella), but occasionally, some members can also cause disease such as *E. coli* 0157. Well-known intestinal pathogens are members of the

Salmonella, Shigella, and Yersinia species. Fecal coliform testing is achieved by increasing incubation temperature to 44.5°C and using different media. Most fecal bacteria cannot multiply in the environment, which is why most evaluations are for total coliform bacteria.

When evaluating coliform bacteria, they are generally grouped as either 'Total' or 'Fecal Coliform' (Water Stewardship Information Series, 2007). The 'Total' group includes Fecal Coliform bacteria like E. coli and others native to soils, which is why it is difficult to base TMDL's on Total Fecal Coliforms found. Due to the difficulties of measuring every potential pathogen (such as parasites), and the issues associated with a correlation of total or fecal coliforms to human health, there has been a push to evaluate numbers of *Enterococcus faecalis* and *E. faecium,* which are bacteria that are normally found in human and other warm-blooded animals' fecal matter. *Enterococcus faecalis* and *E. faecium* can cause human disease such as urinary tract infections, wound infections, endocarditis, and meningitis (the last two are more extreme and more common in hospitalized patients). *Enterococcus* spp. is now identified more often than fecal coliforms, as it is believed to have a higher correlation to the human fecal coliform pathogens often found in sewage (Jin et al., 2004). In addition, *E. faecalis* has a limited host range (so not as common to the natural environment). *Enterococci* can survive salt water; in this respect, they mimic pathogens more so than other indicators, and they are typically more specific to human contamination. Therefore, EPA recommends *enterococci* as the best indicator of risk in recreational salt-water activities, and as a useful indicator for fresh water (EPA, 2011, Water Monitoring and Assessment).

Various techniques exist, and regulators and stakeholders would like to know which techniques provide the most reliable results for a given situation. The main issue is the variety of techniques having varying levels of accuracy and confidence. Another issue in finding 'indicator organisms' is that the indicator bacteria may not only be confined to warm-blooded animals and may be common in invertebrates and sometimes plant material (Ellender, 2002) and to indigenous or migrating bird populations. Most regulators agree that most of the techniques are acceptable under most conditions. However, the more complicated the ecosystem, generally, the more complicated and expensive the evaluations. The science is on the cutting edge and is constantly evolving, especially for molecular techniques and there are still no guidelines for methods to be used in absolutely every scenario. Scientists are still attempting to discover a simple and inexpensive solution that can be applied in all scenarios.

Some obvious bacterial TMDL point sources are storm water, poultry, dairy farming, aquaculture, and other animal operations, municipal, industrial, and pharmaceutical waste. Some less obvious sources are pets, wildlife populations, shore birds and migratory birds, and marine fish. Sometimes a fish kill will occur indicative that something is wrong. This can be due to eutrophication, contaminant loading due to a high nutrient source, or to bacterial contamination. Eutrophication reasons for the fish die off are relatively easy to discern due to commercial off-the-shelf technologies for nutrient sampling.

4.4 EPA RECOMMENDATIONS

The EPA recommends measuring two main indicator organisms: *Escherichia coli* populations in fresh water and *Enterococcus* species (sp.) populations in marine waters. EPA also recommends measuring a broader population of fecal coliforms when evaluating the safety of shellfish harvested for consumption. The concentration of bacterial indicators, *E. coli*, *Enterococcus* sp., and fecal coliforms, can be measured directly through various well-established techniques to obtain a statistical estimate (most probable number) of the bacterial concentration.

Factors influencing pathogen survival once they enter the receiving water body, while potentially numerous, are primarily limited to temperature, salinity, and, most importantly, sunlight (EPA, 2001). The germinal paper relating bacterial die-off rates to these parameters was based on both laboratory and field measurements of coliform populations (J.L.Mancini, 1978). The Mancini model expresses the bacterial die-off rates as functions of light intensity, temperature, and salinity. This model is useful, but more complex models would be more accurate. Coliform bacteria populations were found to exhibit exponential decay in open fresh and marine waters. Fecal coliform bacteria are present in the intestinal tract of animals and generally, die off outside the host within 30 days. However, if the bacteria sink to the bottom of a water body (to escape UV exposure) and are in water bodies that are cooler (again, bottom water is generally cooler than the overlying water column), bacteria can live for a longer time period. In addition, the EPA TMDL protocol cites several studies showing bacteria living several months in sediments, compared to expected persistence of a few days in the water column. Generally, if coliform bacteria are identified over a long period of time, it is presumed that bacteria are continuously entering the water body of concern.

Most sources of bacteria entering a water body are related to rainfall and higher river flow events. Therefore, the time chosen for sampling will be quite important. More often, it is after rainstorms that most places will have issues with excessive bacterial loading. Some of the reasons could be due to the input of storm drains and general run-off. To determine if there is an increase due to rainfall, the results can be calculated by computing the difference in bacterial concentrations before and after a rain effect. Also important is hydrology as it relates to the properties, distribution, and circulation of water on or below the ground water. Hydrology of a receiving stream can change, especially if there is an increased imperviousness, thereby increasing run-off volume and rate.

4.5 LABORATORY METHODS TO TRACK BACTERIAL CONTAMINANTS

Methods to track bacterial contaminants depend on capability and resources. It is possible to differentiate between some animals and humans and work is ongoing to differentiate between sewage treatment plants. On-site, rapid detection techniques are still being evaluated. These rapid techniques provide results that show whether *E. coli* has been detected, but will not discern the *E. coli* source.

No method works under every circumstance and in all media because of the differences between fresh water and marine systems. Investigations are ongoing in various academic and industrial settings to compare and evaluate different MST methodologies. *E. coli* and *Enterococcus* sp. currently have had the most investigations performed, but work is ongoing to investigate other gastrointestinal bacteria. Except for the chemical MST methods, generally all the methodologies require a large library (and a great capacity for statistical analysis of the library). How large the library needs to be to represent different mammalian and avian species and if this library will be representative under all conditions is still unknown. Regardless of all the work still to be performed, we are at the turning point, and it is anticipated that within the next decade, standardized MST methodologies will exist.

Current methodologies often used are Carbon Source Profiling/Community Level Physiological Profiling (CLPP) and Multiple Resistance Analysis. These two methods are less expensive and the libraries are somewhat more established. However, these techniques still have drawbacks that are described in more detail under the Summary of Laboratory Methods section. There are several reports on different MST methodologies used by various agencies. One thorough report by the Washington State Department of Ecology is available

on line at: http://www.ecy.wa.gov/programs/wq/tmdl/sinclair-dyes_inlets/reports-documents.html.

For broken pipes or illicit measurements of illegal connections to storm drains, several methodologies such as smoke and dye tests can be useful. The following information is taken directly from the National Menu of Best Management Practices (http://www.dcr.vi.virginia.gov/soil_&_water/documents/sec-3.pdf).

- Dye Testing. Flushing fluorometric dye into suspicious downspouts can be useful to identify illicit connections. Once the dye has been introduced into the storm system via the connection in question, the water in the collection system is monitored to determine whether an illicit connection is present.
- Smoke Testing. Smoke testing is another method used to discover illicit connections. Zinc chloride smoke is injected into the sewer line and emerges via vents on connected buildings or through cracks or leaks in the sewer line. Monitoring and recording where the smoke emerges, crews can identify all connections, legal and illegal, to the sewer system. Mechanisms on drains should prevent the smoke from entering buildings; however, in some instances, this will occur. It is important to notify the public that the smoke is non-toxic, though it should be avoided as it can cause irritation of the nose and throat for some people.
- Flow Monitoring. Monitoring increases in storm sewer flows during dry periods can also lead investigators to sources of infiltration due to improper connections.
- Infrared, Aerial, and Thermal Photography. Researchers are experimenting with the use of aerial, infrared, and thermal photography to locate dischargers by studying the temperature of the stream water in areas where algae might be concentrated and in soils. It also examines land surface moisture and vegetative growth. This technique assumes that a failing OSDS, for example, would have more moisture in the surface soil, the area would be warmer, and the vegetation
- Visual Inspection. Remotely guiding television cameras through sewer lines is another way to identify physical connections.

4.5.1 When to Sample

In response to contaminants in water, true representation is ideal. Therefore, replicates should be taken from several sites to truly represent the area as a single sample from one point does not represent the entire water body (Wilbur and Whitlock, 2007). Replication should include random samples from the area and, samples should be processed separately for statistical representation (Wilbur and Whitlock, 2007).

Sampling after a rain event(s) should always be included as bacterial numbers will generally be higher; usually due to storm run-off. As a reference, some standards have suggested dry weather sampling is equal to < 0.25 inches of wet fall in the previous 24 hours and wet weather is equal to > 0.25 inches of wet fall in the previous 24 hours. Dependent on results needed, sampling before, during, and after a rain event may be important to establish any differences to help pin-point the variations in contaminant load.

Sampling during or after a rain event is dependent on the contaminant load, the water body and the geological conditions of the area of interest. For example, if dry weather sampling reveals close to threshold numbers, it is likely that immediately after a rainfall, bacterial numbers will likely rise. If the water body is large and fast flowing, then it is more

prone to dilution and vice versa. Some geological properties include areas that are impervious, and often, some bacterial contaminants may be surficial. But during a rain event, these fecal contaminants may wash into the storm drain or water body of interest.

Effective MST requires that appropriate data are collected to meet the objectives of the study. The following is a prime example from the 2005 EPA Guidance document of how the sampling plan should be designed for the study objectives. An analysis indicated cattle as the major source of fecal contamination to a stream on 70% of dates sampled. However, this may not be particularly meaningful if the stream did not exceed water quality criteria on those days. Despite dominance by cattle contamination on most dates, humans could very well be the major source on exceedance dates (post rain event) and, therefore, the logical target of remediation efforts (EPA, 2005).

4.5.2 Library-Dependent and Library-Independent Methodologies

Historically, MST was divided into two broad categories, library-dependent or library-independent bacterial comparisons. Library-independent comparisons are approaches that do not have to rely on resource-rich libraries and databases, but instead rely on host-specific indicators or markers. Library-dependent comparisons rely on site-specific collections of bacterial tests on which unknown bacteria are compared. From a statistical approach, library dependent is a statistical method to identify an unknown bacterium to a known bacterium and library independent generally not requiring a statistical method for source identification, although this is not 100% accurate (Wilbur and Whitlock, 2007).

Library-Independent Methods. Originally, these methods did not rely on resource-rich libraries and databases. However, this is not clear-cut, as library-independent methods can also require developing an initial library of host-specific strains for usage. The technical difference is that these markers can only be strongly associated with specific bacteria from a specific host (Soule et al., 2006). Some examples of library-independent methods are as follows:

- Chemical analysis: caffeine, and optical brighteners
- Bacteroides genotyping
- Enteroviruses
- Specific Coliphage (F+) Analysis
- Multi locus sequence typing (MLST)
- DNA Microarray Analysis
- Desorption Electrospray Ionization (DESI)

Library-Dependent Analysis. Library-dependent comparisons rely on site-specific collections of bacterial test on which unknown bacteria are compared. One challenge is that a large number of isolates must be characterized before a suitable classification equation can be generated. Currently, no set guidelines exist to help determine the number of isolates to characterize per host and how many hosts should be sampled (Johnson et al., 2004). Comparisons can be further divided based on biochemical versus molecular (genetic) tests (Malakoff, 2002). The molecular approaches tend to be more expensive, slower, but more precise, than the biochemical approaches. The EPA has not yet released technical guidance on source tracking technology and its application in TMDL development. A review of the results of some of these techniques and their prevalence

in studies around the country can be found at http://www.epa.gov/reg4gmpo/presentations/bacterial.pdf.

Some examples of library dependent methods are as follows:

Carbon Source Profiling

Antibiotic Resistance profiles

Ribotyping/ DNA Fingerprinting

Randomly Amplified Polymorphic DNA (DNA polymorphisms)

However, because theoretical library-independent methods often rely on some background data (usually a database), these terms were not clearly defined and often confusing. Therefore, a more recent approach is to separate microbial source tracking techniques into chemical, phenotypic (biochemical), and molecular methods. The following sections do not follow the historical terminologies, but follow the more current terminology trends.

4.6 LABORATORY METHODS SUMMARY

Table 3 shows a comparison between chemical, phenotypic (biochemical), and molecular methods. Specifically, each method is summarized into the following categories: library dependent or independent, size of database required, cost, whether methods are accurate and reliable, capacity to discriminate between human and others, maturity level, if the method requires skilled labor, and if the method can be used in freshwater or marine systems. The maturity level is subjective and relative to the other methods on this table. These numbers represent how far along the techniques are and the general scientific community acceptance. At this point, most methods can discriminate between human, livestock, wildlife, and birds. However, even with the best technology, the methods are still not 100% reliable when it comes to discriminating between species. For example, the methods cannot discern horses versus cows or raccoons versus chickens. However, these issues are being addressed and this discrimination should be available within the next 5 years.

4.7 SUMMARY OF RAPID DETECTION TECHNIQUES

Currently, rapid detection methods are unreliable. No technique or instrument can be used to determine the presence and source of bacterial input into the environment. Several techniques can be used as screening methodologies for human or animal input, particularly where there should be none.

Two chemical techniques may be considered rapid in the sense of their simplicity. These are both described in detail below. One technique is the detection of optical brighteners; often common in laundry detergent and indicative of a human input or sewage contamination. Also, desorption electrospray ionization (DESI), is a relatively new mass spectrometric technique for differentiation of bacterial organisms in the laboratory that takes a few minutes and little sample preparation (Yishu Song et al., 2007). However, a significant database for different bacterial contaminants does not exist, and currently, only specific *E. coli* and *Salmonella* sp. can be recognized.

Table 3. Summary comparison between chemical, phenotypic (biochemical), and molecular MST laboratory technologies.

Method	Library (D)ependent or (I)ndependent?	Large database required?	Cost? *	Accurate or reliable?[1] low/med/high	Capabilities?	Maturity Level[2]	Requires skilled labor?*	Fresh water or Marine?
Chemical								
Caffeine	I	No	Med	Med	Human	6.4	High	Both
Optical Brightners	I	No	Low	High	Human	6.4	Low	Both
Desorption Electrospray	I	Yes	High	High	Currently, only few *E.coli* spp	6.1	High	Fresh
Ionization (DESI)					Tested			
Phenotypic (Biochemical)								
F+ Specific Coliphage	I	No	Med	Med	Human vs. other mammals	6.3	High	Both
					Mammals vs. birds			
					Not Human vs. birds			
Carbon Source Community Level Physiological Profiling (CLPP)	D	Yes	Low	Low	Human vs. other mammals	6.4	Low	Both
Multiple Antibiotic Resistance	D	Yes	Low	Low, ~ 50-60% reliable	Human vs. other animals	6.4	Low	Both
Molecular								
Bacteroides Genotyping	I	No	Low	High	Human only	6.4	Med	Primarily fresh water
Enteroviruses	I	No	Med	High	Human only	6.4	High	Both
DNA Fingerprints (Ribotyping)	D	Yes	Med	High	Human and various animals	6.2	High	Both
Multi-Locus Sequence Typing (MLST)	I	Yes	Med	High	Human and other animals	6.1/6.2	High	Both
DNA Microarray Analysis	I	Yes	High	High	Human and other animals	6.2	High	Both

[*,1] The Low, Medium, and High are relative to each other and do not represent any particular number.
[2] The maturity level is subjective and relative to the other methods on this table. These numbers represent how far along the techniques are and the general scientific community acceptance. The maturity level is subjective and relative to the other listed methods. These numbers, 6.1 to 6.5, are how the Department of Defense organizes its research and represents how far along a research technique is; this is often subjective. In general, 6.1 applies to a basic research phase and 6.5 refers to a very well-established and accepted technique. Basic and applied science (largely performed at universities) is 6.1; exploratory development for practical application is 6.2; building of prototypes to demonstrate the principal is 6.3; demonstration and validation are 6.4 levels; and 6.5 is at the engineering and manufacturing development, or, established techniques.

Molecular biology methods are becoming more rapid; particularly for trying to determine fecal coliform contamination,for example, in response to public health issues or beach closures. Generally, these techniques require approximately 4 hours once the samples have been taken into the laboratory. Newer instruments are under development for field sampling, for example, a hand-held thermocycler. However, most of these field thermocyclers are designed to identify biological weapons (such as anthrax, tularemia) and food hazards (such as salmonella or *E. coli* 0157), and, these rapid molecular methods will not usually help determine the source of the fecal contamination. Once the need has been more clearly established, these field samplers can be manipulated to look for DNA specific to the source microorganisms of interest.

4.8 CHEMICAL MST METHODS

Chemical methods do not detect bacteria, *per se*. Historically, these methods were used to identifying human sources. For instance, if synthetic compounds are found in a water body, then a human source is likely. These methods can be useful when one wants to rule out human input (and that may be all that is required). Dye release and smoke tests (mentioned above) are effective for finding broken pipes or illicit input.

4.8.1 Caffeine and Optical Brighteners

Caffeine and optical brighteners (brighteners found in most laundry detergents) are two proxies that can be used for evidence of a human source input. Tests for these two proxies are relatively common. Evaluations for caffeine are not difficult, but usually require a high performance liquid chromatography (HPLC) so costs can run ~ $100 per sample. Moreover, caffeine has a long half-life, so input does not have to be recent (may or may not be important based on the sample); and can be degraded by soil bacteria, so the amount of caffeine present cannot be correlated with human input. Sample collection for brighteners is performed by placing optical brightener-free cotton in a wire mesh trap and placing the trap in the stream for a few days. After the trap is recovered, the cotton is examined with a black light to see if it glows. The fluorescent cotton can then be examined with mass spectroscopy to verify the presence of the compounds. This technique is considered a qualitative measure. However, researchers can obtain some information on the severity and/or primary source of contamination by making note of the relative intensity of each sample's reaction with light when comparing samples exposed for the same amount of time and taken near each other in the same water body.

4.8.2 Desorption Electrospray Ionization (DESI)

A new technique, desorption electrospray ionization (DESI), is a mass spectrometric technique, and is currently being more fully investigated for future usage as a relatively rapid bacterial detection and identification technology (Yishu Song et al., 2007). At this point, once samples are brought into the laboratory, there is little sample preparation and sample analysis can be completed within minutes. This method allows differentiation by direct analysis of ambient bacteria without full sample preparation and allows for “bacterial fingerprinting”. Subspecies of bacteria can be distinguished based on the fatty acids and cell wall components of the bacteria. This technique is a new research field and it is still years out before it can be applied to microbial source tracking, as fingerprints of bacterial communities of interest must first be developed. However, this is an important discovery for microbial source tracking, particularly related to real-time evaluations of bacterial contaminants. Figure 22 and Figure 23 are examples of how different subspecies of *E. coli* and *Salmonella typhimurium* can be

differentiated via the DESI technique and analyzed using principal component analysis. Visually, distinguishing between two different species of *E. coli* and two different sub species of *Salmonella typhimurium* (*S. typhimurium*) is straightforward. Principal component analysis (PCA) score plots are used to help make predictive models to aid in distinguishing different bacterial species. The laboratories perfecting this technique are still in process of building up a database that can be applied on a broader scale.

4.9 BIOCHEMICAL/ PHENOTYPIC MST METHODS

Traditional methods often relied on phenotypic characteristics. However, when using phenotypic methods, it is quite difficult to differentiate similar strains of bacteria from different sources. The usage of phenotypic methods should often be in conjunction with other techniques, such as some of the biochemical ones mentioned below, as phenotypic techniques are generally much less expensive, but less reliable.

4.9.1 F+ Specific Coliphage

A library independent technique is the usage of the F+ specific RNA coliphages. F+ specific coliphages are due to infection of specific bacteria containing the F+ plasmid. The F+ plasmids are circular pieces of DNA that confer fertility; the capacity to conjugate with other bacteria. F+ coliphages are pathogens of E. coli and infect the pilus of male E. coli strains. These coliphages can be differentiated by antigens they produce (serotypes). There are four distinct serogroups of F+ coliphages; those predominating in humans (groups II and III) differ from those predominating in animals (groups I and IV).

Groups I and IV coliphages are generally presumed to be associated only with mammal (not human) and bird fecal material. This is helpful when trying to discern if mammal or bird material is a source; but not when trying to discern between mammals. Many studies and evaluations report that F+ RNA coliphage analysis is used to confirm putative animal and human waste impacts on environmental waters (Cole et al., 2003; Griffin et al., 2004; Brion et al., 2001). At this time, there appear to be some exceptions and a need for caution when using this technique to discern human from animal input as F+ RNA coliphages groups II and III; although usually found in humans, have also been detected in swine (Stewart et al., 2006). However, if swine are not a concern, then this technique is usable.

4.9.2 Carbon Source Profiling/Community Level Physiological Profiling (CLPP)

Carbon source profiling is a technique that generates a profile of a microbial communities based on the source of carbon used by the microbial community. However, this is a culture based assay and to date, best estimates are that only about 5% of the bacterial communities are thought to be culturable. Culture based assays is the taking of different samples (such as water, soil, or sediment) and inoculating selective media to grow up specific organisms.

There are some assays specific for *E. coli* and *Enterococcus*. When the database for different carbon compound utilization is sufficient, *E. coli* and *Enterococcus* bacterial species can usually be determined and differentiated. These bacteria have been established as indicator organisms representative of fecal contamination. Unfortunately, there is no magic number for a ‘sufficient’ library. Some watersheds have an extensive library; and in these situations, it may be possible to differentiate between human and other profiles. However, one drawback is that the physiology of the bacteria can change dependent upon the environment. For example, *E. coli* in the animal gut have a different profile than that under environmental conditions (Joyce Simpson et al., 2007).

The profile is generated by using commercially available microplates, containing as many as 95 different carbon sources (Figure 24). The approach is based on measuring a variety of carbon sources that can be utilized by the bacteria as food sources. Carbon utilization profiles are easy to perform and interpret; relatively inexpensive and there is automated analysis available. However, this is also culture dependent, and often ecologically irrelevant.

Carbon utilization assays are performed using diagnostic kits where the biochemical and phenotypic characterization occurs, as a means for a metabolic "fingerprint". Essentially, bacterial cultures are placed into several wells containing specific sugars or carbon compounds that can be used for growth. Some examples of the sugars in the wells are ethanol, glucose, lactose, glycogen, xylose, serine, pyruvic acid, etc. Bacteria generally cannot oxidize (use) all carbon sources, but knowing which wells are positive (where there is growth), will provide clues as to the physiology and the species of the bacteria.

This method is growing in usage, but there still are setbacks in that this method is library dependent and requires statistical analysis to truly be able to source bacteria to point of origin. This method is shown in Figure 24. Despite the drawbacks, this may be one helpful method for microbial source tracking. This would provide better data if used along with a different method (i.e., Antibiotic Resistance Analysis), for better results. If a year-long study were to be performed at a particular site, better results would be to incorporate this method with the ARA method when establishing the library of different sources of interests.

4.9.3 Multiple Antibiotic Resistance

The antibiotic resistant analysis (ARA), sometimes called multiple antibiotic resistance (MAR), is the often-used method of microbial source tracking as it is relatively inexpensive. Generally, certain animals (livestock, wild life, and humans) tend to have normal flora that are resistant to certain types of antibiotics (Wiggins et al., 2003d) and it is believed that bacteria from humans are more sensitive to antibiotics. When bacteria are subjected to a new antibiotic; most will succumb (dependent on the antibiotic and outer envelope or cell wall component of the bacterium). Over time, some bacteria will spontaneously mutate and develop a resistance to the antibiotic; thus, the specific resistance of the bacteria and/ bacterial population changes over time. Therefore; this technique cannot be guaranteed long-term due to the potential changes in antibiotic resistance. One specific example concerns dairy cows exposed to antibiotics, and perhaps on the same farm, an organic, no antibiotic dairy cow herd. When attempting to discern these dairy cows from other potential sources, the bacteria from the gut of the cows constantly exposed to antibiotics in the dairy farm will be very different from the unexposed cows and there will be two different ARA results from the cow fecal matter, making it more difficult to distinguish the source.

Other studies indicate that a more robust ARA library size does not always contribute to an easier analysis. One study compared antibiotic resistance analysis versus ribotyping for identification of fecal pollution sources in a watershed (Moore et al., 2005). In this study, by Moore (2005), libraries were constructed from several sources: seagulls, dogs, cats, horses, and humans, and results showed that the accuracy of placing the source via the ARA technique was only 28% for *E. coli*, and 48% for *Enterococcus*. This was true even when ~2500 more *E. coli* isolates were added to the library. No one can explain why *E. coli* fared worse than *Enterococcus*. A study by Wiggins, et al., (2003) had a library of over 6000 A study by Wiggins, et al. (2003) had a library of over 6000 isolates and even with a library of that magnitude, suggested this would likely only be representative of an ARA profile for 1

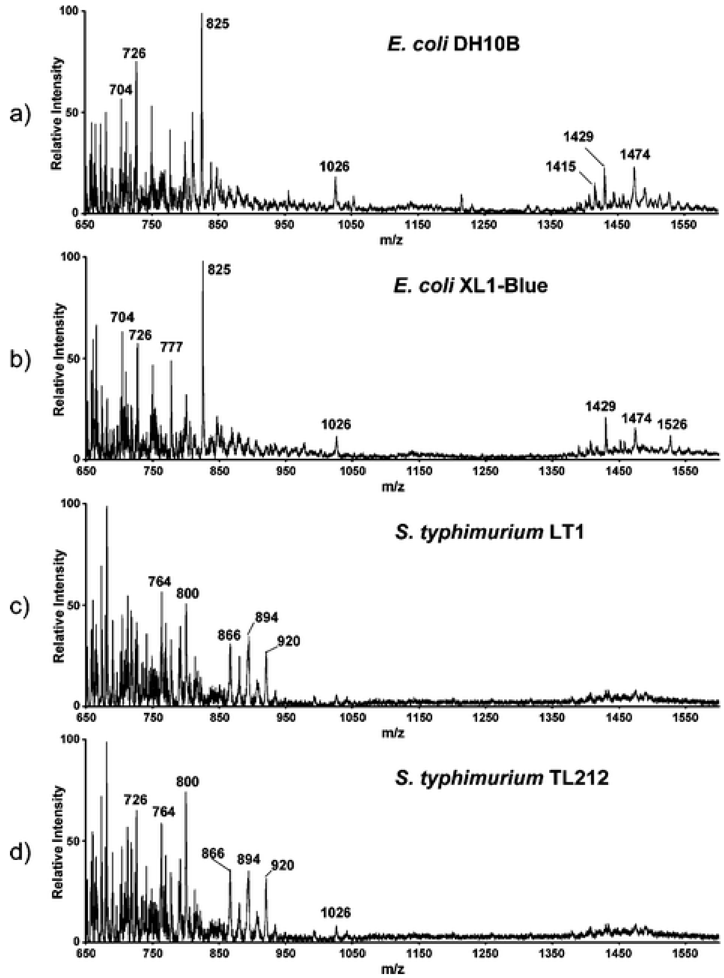


Figure 22. Typical DESI mass spectra of a)*E. coli* DH10B, b)*E. coli* XL1-Blue, c)*S. typhimurium* LT1, and d)*S. typhimurium* TL212 (Song et al., 2007).

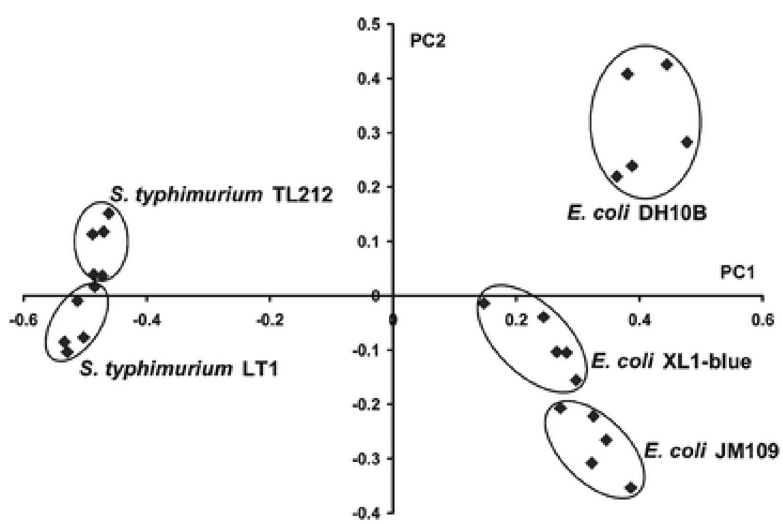


Figure 23. Principle component analysis (PCA) score plot of five strains of bacteria cultivated and analyzed under identical conditions using the DESI technique(Song et al., 2007).

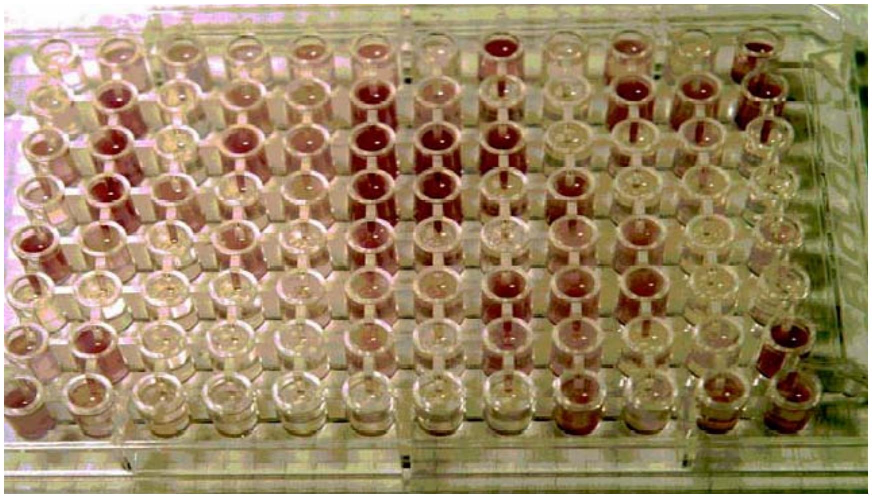


Figure 24. Microtiter plate shows community-level physiological profiling. (Garder, 2003) The dark color (in this case, purple) in the wells indicates that bacteria can use and grow on the carbon sources available in the well.

year and, that at least 2300 isolates are required for a representative library (Wiggins et al., 2003c). The conclusion from the Moore (2005) and Wiggins papers (2003) indicate that the library based ARA method may not be suited for determination of the fecal pollution source. The average rate of correct classification is calculated by self-crossing the database and determining the mean that the classification is correct. Also and not intuitive, it can be misleading to only rely on the average rate of correct classification (ARCC) because the larger the library, the lower the ARCC. This is primarily because there is a larger library and there will be a higher number of unknown isolates (Wiggins et al., 2003b). One reason for the differences in bacterial resistance patterns may be due to different usage in antibiotics and also, bacteria are constantly changing with regards to antibiotic resistance. Bacteria can adapt and become more or less susceptible to specific antibiotics. Studies have been performed and shown to be reliable up to one year (Wiggins et al., 2003), and in the best case, most will be reliable a little longer.

Unfortunately, sometimes it is necessary to have better clues as to the source for pollution such as fecal coliforms. The ARA technique is one of the least expensive and therefore, is commonly used. Therefore, to obtain better results, it may be better to perform more samples, but to analyze fewer categories as a work-around. References have demonstrated that the bigger the library size, the better the results. Unfortunately, there is no sure number to determine if the library size is large enough. As one study above indicated, a library of 6,000 seemed to help. However, this may be impossible. One suggestion is to try to establish the library with as many samples as financially feasible. Another suggestion to improve statistics is to analyze more than the suggested 12 samples per study area when doing the actual sampling.

4.10 MOLECULAR MST METHODS

Molecular approaches to environmental management are becoming more common, especially as it pertains to real-time analysis. The Southern California Coastal Water Research Project (SCCWRP) is working with the California Department of Health Services (DHS) to develop a laboratory certification process for many of these new methods. DHS presently has a laboratory certification process for biochemical and phenotypic methods, but

does not have a similar certification for molecular methods. Molecular methods present new quality assurance (QA) challenges. For instance, disinfection procedures that are routinely applied with existing methods, such as using alcohol to clean equipment between samples, will be ineffective at removing remnant genetic material and can lead to sample cross-contamination. However, with time and decreasing cost, these molecular methods will likely be the primary methods used for real time and general microbial source tracking.

Due to the popularity and clarity provided by Multi Locus Sequence Typing (MLST) and the DNA Microarray analysis, we expect these techniques to be worked and examined extensively within the next few years and within 5to 10 years. Guidelines will be established after sufficient peer review, for the usage of MLST and DNA Microarray analysis to track fecal contaminant sources.

4.10.1 Bacteroides genotyping

Bacteriodes genotyping is dependent on identification of a bacterium that is only found in the mammalian colon. This method involves genetic identification via polymerase chain reactions (PCR) of an obligate anaerobic bacterium not related to *E. coli* that lives primarily in the mammalian colon. This technique is useful when attempting to determine if there is a potential human source. However, new research methods are currently under investigation to quantitatively distinguish mixed sources such as human, cow, and dog; and also, qualitatively distinguish pig, chicken, and elk (Bambic, Carlson, and Baharians, 2007). All of these samples are taken from fecal material.

4.10.2 Enteroviruses

Enteroviruses are viruses that may be associated with the intestine, and some are pathogenic. Studies exist that attempt to use this technique for MST, and these studies are similar to measuring for indicator bacteria. For example, human-associated viruses called enteroviruses or adenoviruses, bovine (cow)-associated enteroviruses, and porcine (pig) teschoviruses that generally only infect the associated hosts (Stewart, 2006). The information collected from analysis of these viruses can provide specific information as to a potential source of contamination.

Several known human-only pathogenic viruses can be identified: HIV-1 (human immunodeficiency virus), HTLV-I and -II (human T-cell leukemia/lymphoma virus), EBV (Epstein-Barr virus), HBV (hepatitis B virus), HCV (hepatitis C virus), and HHV-8 (human herpesvirus-8, Kaposi’s sarcoma-associated herpes virus) are all some examples. Obviously, not all humans are carriers of these viruses, so even if the test is negative, human feces still may be present. Molecular techniques are used to identify these enteroviruses via polymerase chain reaction (PCR) or RT-PCR (reverse transcriptase-PCR) for detection of specific DNA or RNA sequences of these human pathogenic viruses. However, the material is still developing for this methodology, specifically concerning other non-human sources.

4.10.3 Randomly Amplified Polymorphic DNA (DNA polymorphisms) (RAPD)

DNA polymorphisms are one of two (or more) alternate forms (alleles) of a chromosomal locus that differ in nucleotide sequence or have variable numbers of repeated nucleotide units. In layman’s terms, a condition in which one of two different, but normal nucleotide sequences can exist at a particular site in a DNA molecule. The RAPD methodology involves identifying unique polymorphisms within the DNA of fecal bacteria. Arbitrary primers are used to identify randomly selected polymorphisms, and amplification occurs via polymerase

chain reaction (PCR). DNA is isolated and amplified using specific primers and evaluation by gel electrophoresis. However, this method requires screening of primers (> 1500 commercially available) to find sets of polymorphisms that are unique to fecal bacteria from a specific source. However, once specific primers for DNA polymorphisms are found, fecal bacteria can be "sourced" by comparison.

4.10.4 DNA Fingerprints (Ribotyping)

Ribotyping is an electrophoresis banding pattern of conservative genes coding for ribosomal ribonucleic acids (rRNA). This method is likely the method of choice as it is generally much less expensive than other sequencing techniques. However, this technique requires a more detailed analysis and expertise.

The ribosome is the cell structure where proteins are manufactured and ribosomal genes are highly conserved in microbes, meaning that the genetic information coding for rRNA will vary less within bacteria of the same strain than between bacterial strains. This characteristic allows for a greater ability to distinguish between different bacterial strains and looking at small differences in the DNA help identify different strains of E. coli. Figure 25 depicts a cartoon courtesy of the Southern Regional Water Program of how ribotyping functions (SRWQIS, 2007). The technique used is terminal restriction fragment length polymorphism (t-RFLP) and evaluates patterns of fragment lengths of enzyme-digested rRNA.

In ribotyping, restriction enzymes are used to cut the genes coding for the ribosome and electrophoresis separates the pieces by size through a gel. Genetic probes then visualize locations of different-size fragments of DNA in the gel, which appear as bands. The banding pattern of DNA fragments corresponding to the relevant rRNA is known as the ribotype. The banding patterns are compared to a database of other E. coli strains and matched for each determined strain.

Ribotyping is a relatively inexpensive technique useful for identifying genotypic differences between human and various animal indicators. Often, however, samples are obtained from within confined geographic sites and are watershed specific (Scott et al., 2003) and libraries need to initially be established prior to utilizing this technique. Studies were performed to determine if ribotyping could be applied to discriminate between human and non human (Salina Parveen et al., 1999b), and if they could be universally and geographically applied (Scott et al., 2003). Both studies indicate that differences between animal and human could be distinguished universally (Salina Parveen et al., 1999a; Scott et al., 2003), but not differences among animals, i.e., seabirds, swine, poultry, beef, or dairy cattle (Scott et al., 2003; Moore et al., 2005). Moreover, the overall miscalculation rate among the animals tested from different geographical locations was 65.3% (Scott et al., 2003). A separate study using a library of 4,500 organisms (from humans, dogs, cats, horses, and humans) found only an average rate of correct classification (ARCC) of 69 % for *E. coli* (Moore et al., 2005). These data strongly suggest that ribotyping is a feasible alternative for microbial source tracking for the water program manager prospective if there is only the need to distinguish human *E. coli* from animal *E. coli* contamination. However, if there is a need to distinguish amongst animals, ribotyping is currently not the best strategy.

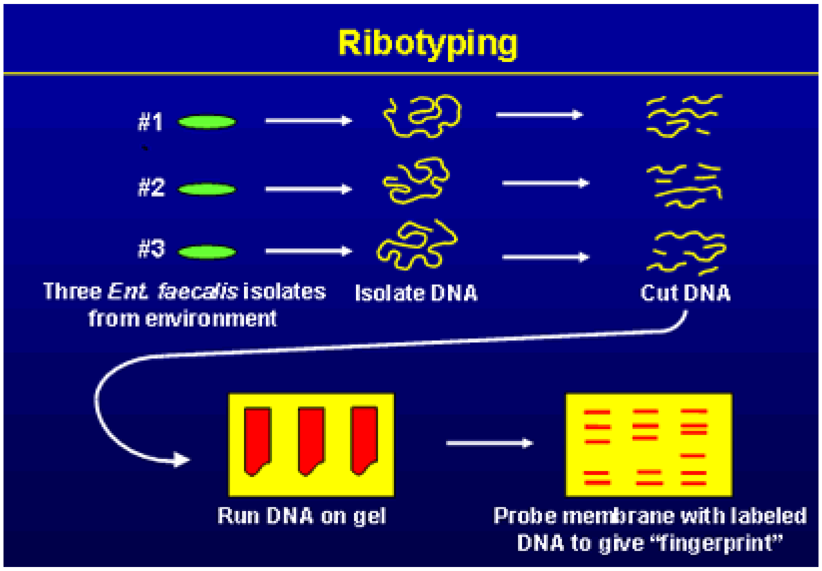


Figure 25. Diagram showing how ribotyping functions.

4.10.5 Multi locus sequence typing (MLST)

Multi locus sequence typing (MLST) is a simple technique of DNA analysis that is used for the characterization of multiple loci. A locus is a fixed position on a chromosome; such as the position of the gene within the chromosome. Therefore, one technique to identify bacteria is to isolate bacterial DNA and to sequence a few housekeeping genes that all bacteria have that are 'always on'/ constitutively expressed and are needed for general cell maintenance. MLST is a method that uses and is reliant on the more conserved part of the genome and indexes the variations within this conserved region (Thakur et al., 2006). In MLST, small fragments of the DNA are sequenced and the different housekeeping genes are assigned to a distinct allele. Alleles that are positive will provide an allelic profile or sequence type (ST) that is specific to each bacterium and this information will provide a phylogenetic fingerprint of bacteria present. MLST was initially proposed as a simple and definitive mechanism to characterize bacteria (Maiden et al., 1998) and data and libraries are available via the internet; particularly for pathogenic species of bacteria (Maiden, 2006). Unfortunately, this technique has not been extensively used and there is basic applied research on-going to differentiate cattle, chicken, and swine Campylobacter strains (Miller et al., 2006). However, once a basic library has been established, fluorescent in situ hybridization (FISH) can be performed on many samples at a time and determine quantification when searching for particular bacteria in mixed samples; for example, *E. coli* from human, poultry, or swine. FISH allows the usage of fluorescent probes to bind to specific parts of the chromosome (or specific loci). This is shown in Figure 26. MST analysis in conjunction with FISH is quite likely the future direction for bacterial source tracking to differentiate bacteria from host strains simultaneously.

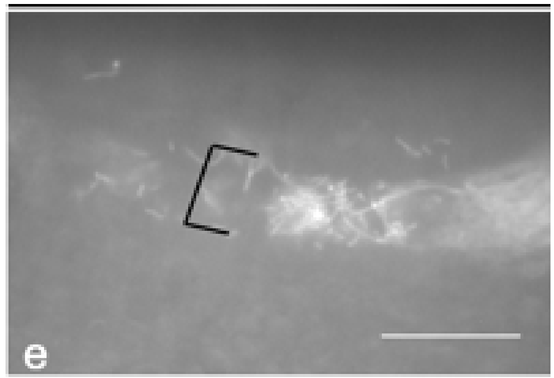


Figure 26. Example of how the FISH technique can be used to stain specific bacteria. In this case, a specific symbiotic bacterium was targeted and identified within a specific area (pallial sinus) in *Bugula neritina* (bryozoan invertebrate that resembles moss) larvae. Close-up view of a FISH hybridization of a larva with Eub338 (a bacterial primer used to detect bacteria cells). The location of the pallial sinus is indicated by brackets. Scale bar, 5 µm. (Lim and Haygood, 2004).

4.10.6 DNA Microarray Analysis

DNA Microarray Analysis is currently under investigation as a mechanism to track microbial sources without the necessity of library classification procedures. Investigations were constructed to try to differentiate between cow, dog, elk/deer, human, and waterfowl (Soule et al., 2006). This method attempted to identify library-independent markers to discriminate between animal and human fecal contamination sources. Despite the positive results for distinguishing between different contaminant sources; several challenges still exist, and the methods are tedious (Soule et al., 2006). However, one recommendation is to use a simple absence/presence detection through multiple site visits. For example, conclusions would be based on positive events rather than investing considerable effort to carefully enumerate individual single samples. Quoting from Soule et al., 2006, "…if a site is visited ten times, and on three occasions markers are detected for human feces, but cattle fecal markers are detected for all 10 samples, then one can draw the reasonable conclusion that the contribution from cattle is the first concern."

4.11 DATA ANALYSIS

Statistical analysis in microbial source tracking is an important issue. A chapter has been devoted to this subject alone in the book, *Microbial Source Tracking* (Wilbur and Whitlock, 2007), and a brief summary is presented here. Due to the differences of some methods having more specificity but less sensitivity, and vice versa, when possible, it is always best to use an approach that incorporates at least two, if not several, methods to determine validity of results (and negate false positives/negatives).

Methods for sampling to facilitate better data are to obtain a random sample of test isolates and many samples over a temporal period (including rainy periods). 'Many' is a relative term and is dependent on the methodology used. For example, when trying to establish absolute differences between swine, horses, and cows, and using the ARA technique, 20 samples from one representative animal would probably not be sufficient. The more samples obtained and the more animal representatives, the more valid the results. The number of samples is dependent on the objective.

Cross-classification is also a helpful methodology to determine the rates for correct classification (RCC). This is a technique where the number or source isolates correctly classified is divided by the number of total source isolates. The result is the probability that the method will correctly identify future environmental samples (Wilbur and Whitlock, 2007). Table 4 is an example of a table used to determine the RCC. Using Table 4, one example is to examine the data for the origin of cow, where 467 samples were analyzed and cow was correctly identified 321 times (69%). However, even though the origin was cow, the answer came up as dog, 54 times (12%); human, 9 times (2%); and wildlife, 0 times (0%). In 83 instances (18%), identification was not established. In this example, you can determine the lower and upper confidence limit; these have to compare to the risk the investigator is willing to allow. For more information on how these numbers are used and the math involved, please see the chapter written by Wilbur and Whitlock (2007).

Table 4. Results of cross-classification for Whitlock data taken from Wilbur and Whitlock (2007).

Origin	Classification of Isolates				
	Cow	**Dog**	**Human**	**Wildlife**	**Total**
Cow	321	54	9	0	n1= 384
Dog	38	333	57	52	n2= 480
Human	102	195	702	169	n3= 1,168
Wildlife	6	48	169	231	n4= 366
	n*1= 467	n*2= 630	n*3= 849	n*4= 452	n= 2,398

Discriminant analysis is another statistical method used to predict into which of two or more groups an unknown sample will belong. Several software packages have been used in microbial source tracking. Just a few mentioned here are MATLAB, BioNumerics system and DiversiLab, the last two incorporating tools for image analysis, data management, and discriminant analysis (Wilbur and Whitlock, 2007).

Temporal and spatial variation is important as it will be rare that indicator bacteria will cover an entire geographic range. Many microbial source-tracking studies will often develop libraries from isolates near the area sampled. A study by Wiggins et al. (2003) found that geographically small libraries (i.e., from specific watersheds) had high rates of correct classification, but were less able to correctly classify isolates outside of the geographic area. Promising results from this study (Wiggins et al., 2003a) are that merged multi-watershed libraries were created and found to be reliable for up to 1-year post library input; indicating temporal stability. This is particularly promising for a central repository for molecular biology libraries suggested below in Section 4.12.

4.12 TECHNOLOGY GAPS

Currently, most MST analysis has only been tested in a limited number of watersheds (Simpson et al., 2007) and over broad geographic areas. Currently, many people are performing antibiotic resistance analysis (ARA) studies. Unfortunately, drawbacks exist over time, and antibiotic resistance in bacteria changes. This is especially true when working in watersheds near non-organic farms as antibiotics are used in both plant and animal agriculture. With greater exposure to antibiotics, the more rapid bacteria will change their antibiotic resistance profile, making the ARA less useful. A long-term database that can be easily shared is one recommendation to make the ARA more useful.

A chemical method, desorption elecrospray ionization (DESI), appears to have the greatest potential for rapid resolution of bacterial contamination. With further investigations, this technique should be able to determine rapidly if bacteria of interest (the initial goal will be pathogens) are present in the sample. Expanding the database and ensuring reproducible and accurate results is time-consuming. However, since more laboratories are investigating this technique, this database should be established faster for bacteria from different sources.

Molecular techniques are more likely to be the primary tool for performing MST since molecular biology is more common, reagents and methodologies are rapidly becoming less expensive, and increasing publications are establishing a greater MST library at various watersheds. At this point, the molecular MST libraries are the limiting factor in greater usage of these techniques. A central repository for molecular library data is likely the biggest technology gap. Once a repository is established, data can be organized by molecular technique, watershed, and comments gathered as to ease of use, reliability, and reproducibility. The key is to determine the manager of such a library. A repository could be as simple as the establishment of a Wiki for collaborative access.

Multi-disciplinary research teams are critical to help discriminate between different pollution sources (Simpson et al., 2007). Hydrodynamics and geology are also important to understanding potential point sources. Hydrodynamics is the study of fluid motion and this can be important when following a BST or TMDL trail, especially if TMDLs are in exceedance after rain or ‘wet’ events. Geology from this perspective is the understanding of the terrain in question. For example, is the terrain composed of clay or sand? This will help in understanding the affect that hydrodynamics may have on the movement of bacteria (or other sources of interest).

4.13 CASE STUDIES

Gauthier et al. (2006), in the Navy TDML Technical Guidance document, analyze several case studies for microbial total maximum daily load (TMDL), but they will not be repeated here since they do not specifically use MST. Some of these TMDLs could apply to forensic MST investigations. Washington State Department of Ecology has a detailed MST study for the Sinclair/ Dyes Inlets Water Quality, which is accessible online at http://www.ecy.wa.gov/programs/wq/tmdl/sinclair-dyes_inlets/reports-documents.html. A recent case study of a microbial source-tracking project in the Virginia Beach Coastal Area of the Chowan River Watershed at NAS Oceana, Virginia, is included in Appendix B.

4.14 CONCLUSIONS

Considerable resources are expended to reduce bacterial contamination in watersheds, but in many cases, storm drains continue to discharge large concentrations of fecal indicator bacteria (FIB). One plausible explanation for the ubiquitous nature of FIB from storm drains is that they survive for extended periods, or perhaps even grow in beach or storm drain sediments. Early evidence by SCCWRP and others has shown that regrowth in sediments is plausible. Beach sediments can be amenable to the regrowth of bacteria, with a constant supply of bacteria from roosting birds and organic media in the form of stranded drift seaweed and kelp.

The methodologies aforementioned for microbial source tracking are only a subset of the most common and more recent developments. Microbial source tracking is a growing area. Currently, no method is preferred for microbial source tracking, as there are pros and cons to the current methods.

More MST research is needed to examine the population dynamics of fecal indicator organisms, the time that these indicator organisms remain unchanged, and the reliability of the various methods. Once a shared database is established, it may move the research community more quickly into resolving the best and most efficient methodologies needed for the field of forensic microbial source tracking.

5. ORGANIC SOURCE TRACKING

5.1 INTRODUCTION

Compliance programs need to determine the sources of organic contaminants at Navy sites, and thus need to review the forensic techniques that can be used to determine these sources. For Navy Compliance programs, the Navy TMDL Prioritization Report (NAVFAC, 2006) lists the main contaminants of concern for that program, and polychlorinated biphenyls (PCBs) tops the list, as stated in Section 1. Unfortunately, many of the analytical techniques developed for these types of regulatory programs do not provide the level of detail needed for forensic studies, so there is a need for this Users Guide, which details the analytical requirements for forensic type studies. A similar previous Navy report (Stout et al., 2003) reviewed the forensic techniques available for polyaromatic hydrocarbons (PAHs), a commonly found organic contaminant at Navy facilities. This document will continue those efforts and focus on reviewing techniques employed for fingerprinting PCBs, another common organic contaminant found at Navy facilities. Although pesticides are not a major contaminant of concern (COC) at most Navy facilities, they are still occasionally present, and many of these PCB techniques can be employed to identify pesticide sources as well.

PCBs are not a single compound, but a class of chlorinated organic compounds that consist of a basic biphenyl backbone with substitutions of from 1 to 10 chlorine atoms. Although there are 209 possible unique ways in which these chlorines can be substituted onto the 10 possible sites on the biphenyl rings, in practice, there are only about 100 individual PCBs (termed congeners) that are normally measured at significant concentrations in forensics studies. This is because PCBs have historically been produced and released into the environment as a limited set of a few very distinct mixtures of congeners, termed Aroclors. PCB Aroclors were manufactured for these specific industrial uses in the U.S. from the 1930s until the 1970s when production was outlawed. Many uses continue today, so releases to the environment are still occurring. But even without fresh sources, PCBs are long-lived and due to a lipophilic nature (tend to concentrate in fatty tissues), they tend to bioaccumulate up the food chain. They are an important environmental and human health concern, and medical studies show cancer risks with higher levels of PCBs, and even at low levels, decreased learning potential in children.

PCBs can be measured by a number of different techniques depending on the intended use for the resulting data. These techniques range from simple, rapid methods such as immunoassays that can provide total PCB levels (including near real-time data in the field) to more complete congener methods using laboratory Gas Chromotography separations with Mass Spectroscopy (GC/MS) detection. In-between these extremes are simple laboratory methods that determine total Aroclor levels by quantifying several key congeners. Although there was some overlap in the use of some congeners in the various Aroclor formulations, it only requires relative compositions of 5 to 10 key congeners for each Aroclor to reveal an unaltered Aroclor pattern. Unfortunately, in environmental samples, multiple Aroclor sources may be present and these source signatures may be altered by a number of natural processes once released into the environment. So to determine the number of original Aroclor sources that may have been present, it may be necessary to unravel the alterations that may be "hiding" the original Aroclor patterns. Forensic studies may therefore need to use a combination of methods to achieve their goals (Stout et al., 2003), which may range from discriminating sources for initial source control to later use for remedial cost apportionment. Thus, a basic knowledge of PCB source, alteration, and bioaccumulation chemistry will

therefore be required. The following sections of this document provide a brief discussion of these PCB chemistry issues prior to the discussion of the analytical methods, data analysis, and case studies.

For a more complete review of PCBs issues, many good online references are available that address various issues. One good general reference is the 2001 National Research Council (NRC) report, “A Risk Management Strategy for PCB Contaminated Sediments,” which addresses sediment issues including identifying sources (http://books.nap.edu/openbook.php?record_id=10041&page=R1).

For review of how forensic studies fit into a regulatory programs, one can view various studies on the Hudson River (http://www.epa.gov/hudson/background.htm) for review of how fingerprinting fits into a cleanup program. For regulatory programs on the compliance side, forensic studies are part of identifying sources under TMDL programs and review of the Delaware River PCB TMDL is a good example of an overall TMDL program (http://www.epa.gov/reg3wapd/tmdl/pa_tmdl/delaware%20river/index.htm) that includes identifying sources. Much of the discussion in this review can also be found on the ESTCP website under the final report for Project ER0826 (Leather et al., in prep).

5.2 CHEMICAL NATURE, HISTORY OF USE, RELEASE, AND ALTERATION IN THE ENVIRONMENT

All PCBs were intentionally manufactured through direct chlorination of the biphenyl molecule (two 6-carbon phenyl rings bonded together) for specific industrial purposes. This chemical process places from 1 to 10 chlorines on available biphenyl locations at the “corners” of the six-sided biphenyl rings (see Figure 27). As shown in Table 5 (originally from Johnson et al., 2006), multiple naming conventions have developed to differentiate the 209 possible congeners, or unique combinations of attached chlorines. Early naming conventions relied on substituted chlorine positions to differentiate congeners. These early methods numbered the six positions of carbons in each ring in the biphenyl structure, and referred to the individual congener by the numbers where substituted chlorines resided (for example, see Figure 27, where ortho positions are labeled 2,6,2’,6’; meta positions are labeled 3,5,3’,5’; and para positions are labeled 4 and 4’). Later, the International Union of Pure and Applied Chemistry (IUPAC) naming conventions simplified things by sequentially numbering all congeners by increasing chlorine content, from PCB 1 through PCB 209. PCBs can also be simply divided into homolog groups based upon molecular weight, distinguished easily as ten distinct classes by different numbers of chlorines substituted onto the biphenyl rings (mono-, mi-, tri-, tetra-, penta, hexa-, hepta-, octa-, nona-, and deca-chlorobiphenyl). These various naming conventions are all shown together in Table 5.

PCBs were produced commercially in the U.S. from 1929 through 1977 by several companies (Holoubek, 2001). They were produced in very specific congener mixtures (trade-marked as Aroclors) to obtain specific chemical properties that were desired for specific industrial applications. Each Aroclor mixture was a unique combination of about 20 to 50 individual congeners, formulated to provide specific chemical properties. PCBs were designed to provide certain required chemical properties to meet specific industrial needs. With increasing levels of chlorination, PCBs show increasing density, boiling point, hydrophobicity, and can be tailored with comparable ranges in many other useful chemical properties. High molecular weights and boiling points lead to a viscous fluid with low flammability that can tolerate high temperatures without substantial chemical breakdown. Due to these useful chemical properties, most PCBs were produced for use in transformers

and capacitors, with other uses including hydraulic fluids, carbonless copy paper, printing inks, paints, insulation, and other applications (de Voogt and Brinkman, 1989). When manufactured in the U.S. by Monsanto Chemical Company, these mixtures were termed Aroclors, while those manufactured outside the U.S. carried trade names such as Clophen (Germany), Prodolec (France), and Phenoclor (Japan). Monsanto is reported to have produced from 500,000 to 600,000 metric tons of Aroclor (about half the worldwide total) during production (Holoubek, 2001), much of which is still in use today and potentially still available for release to the environment. For purposes of simplicity, commercial PCB mixtures will be referred to throughout this report as Aroclors.

Only a limited set of Aroclors were produced in the U.S., each with a distinct congener fingerprint. Aroclors carried a four digit numbering convention, with the first two digits representing the product molecular size and the last two digits representing the weight percent chlorine. For example, Aroclor 1260 has “12” for the 12 carbons in the biphenyl rings to indicate it is a PCB product, and the “60” indicates the mixture of congeners was selected to provide a mixture that has 60% chlorine by weight. Given that only a few distinct Aroclors were produced, and their generally stable chemical structure, one might assume that fingerprinting the distinct Aroclor sources should be a relatively easy exercise. Figure 28 shows some typical Aroclor patterns that can be discriminated even with only the subset of 18 NOAA Status and Trends Program congeners plotted out on a relative weight basis. NOAA selected these congeners because they represent the most commonly found congeners in the environment, and this group often represents about 30-50% of the total Aroclor content in environmental samples. This subset of 18 congeners is often the minimum number of congeners measured in environmental studies, but as discussed in the following paragraphs, it may not be sufficient for forensics studies. The last example shows an equal mixture of Aroclors 1254 and 1260, which might represent the bar chart “fingerprint” of an environmental sample that had a source of PCB with equal amounts of unaltered Aroclor 1254 and 1260.

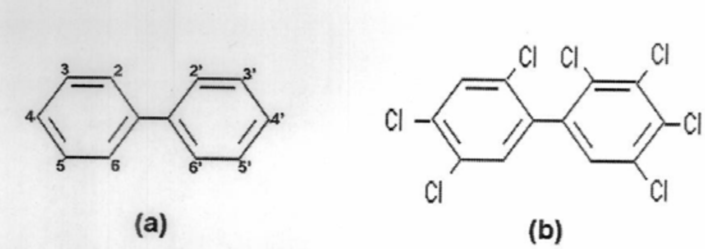


Figure 10.1.1 *Polychlorinated biphenyl molecule. (a) shows a biphenyl molecule showing the ten possible locations where a chlorine atom may be attached to the molecule. (b) shows a PCB molecule with chlorine substitution at the 245 locations on one phenyl ring, and at the 2,3,4,5 locations on the second phenyl ring. This particular PCB congener is referred to as 245-2′3′4′5′ CB or alternatively, PCB 180 (IUPAC nomenclature). Positions 2,6,2′, and 6′ are “ortho” positions. Positions 3,5,3′, and 5′ are “meta” positions. Positions 4 and 4′ are “para” positions.*

Figure 27. Example of PCB congener chemistry (from Johnson et al., 2006).

Table 5. All 209 PCB congeners and various naming conventions.

IUPAC	Structural (Chlorine Pos)
Mono-Chlorobiphenyls	
1	2
2	3
3	4
Di-Chlorobiphenyls	
4	2-2′
5	23
6	2-3′
7	24
8	2-4′
9	25
10	26
11	3-3′
12	34
13	3-4′
14	35
15	4-4′
Tri-chlorobiphenyls	
16	23-2′
17	24-2′
18	25-2′
19	26-2′
20	23-3′
21	234
22	23-4′
23	235
24	236
25	24-3′
26	25-3′
27	26-3′
28	24-4′
29	245
30	246
31	25-4′
32	26-4′
33	34-2
34	35-2′
35	34-3′
36	35-3′
37	34-4′
38	345
39	35-4′
Tetra-chlorobiphenyls	
40	23-2′3′
41	234-2′
42	23-2′4′
43	235-2′
44	23-2′5′
45	236-2′
46	23-2′6′
47	24-2′4′
48	245-2′
49	24-2′5′
50	246-2′
51	24-2′6′
52	25-2′5′
Tetra-chlorobiphenyls (Cont.)	
53	25-2′6′
54	26-2′6
55	234-3′
56	23-3′4′
57	235-3′
58	23-3′5′
59	236-3′
60	234-4′
61	2345
62	2346
63	235-4′
64	236-4′
65	2356
66	24-3′4′
67	245-3′
68	24-3′5′
69	246-3′
70	25-34′
71	26-3′4′
72	25-3′5′
73	26-35
74	245-4′
75	246-4′
76	345-2′
77	34-3′4′
78	345-3′
79	34-3′5′
80	35-3′5′
81	345-4′
Penta-chlorobiphenyls	
82	234-2′3′
83	235-2′3′
84	236-2′3′
85	234-2′4′
86	2345-2′
87	234-2′5′
88	2346-2′
89	234-2′6′
90	235-2′4′
91	236-2′4′
92	235-2′5′
93	2356-2′
94	235-2′6′
95	236-2′5′
96	236-2′6′
97	245-2′3′
98	246-2′3′
99	245-2′4′
100	246-2′4′
101	245-2′5′
102	245-2′6′
103	246-2′5′
104	246-2′6′
105	234-3′4′
106	2345-3′
Penta-Chlorobiphenyls (Cont.)	
107	234-3′5′
108	2346-3′
109	235-3′4′
110	236-3′4′
111	235-3′5′
112	2356-3′
113	236-3′5′
114	2345-4′
115	2346-4′
116	23456
117	2356-4′
118	245-3′4′
119	246-3′4′
120	245-3′5′
121	246-3′5′
122	345-2′3′
123	345-2′4′
124	345-2′5′
125	345-2′6′
126	345-3′4′
127	345-3′5′
Hexa-chlorobiphenyls	
128	234-2′3′4′
129	2345-2′3′
130	234-2′3′5′
131	2346-2′3′
132	234-2′3′6′
133	235-2′3′5′
134	2356-2′3′
135	235-2′3′6′
136	236-2′3′6′
137	2345-2′4′
138	234-2′4′5′
139	2346-2′4′
140	234-2′4′6′
141	2345-2′5′
142	23456-2′
143	2345-2′6′
144	2346-2′5′
145	2346-2′6′
146	235-2′4′5′
147	2356-2′4′
148	235-2′4′6′
149	236-2′4′5′
150	236-2′4′6′
151	2356-2′5′
152	2356-2′6′
153	245-2′4′5′
154	245-2′4′6′
155	246-2′4′6′
156	2345-3′4′
157	234-3′4′5′
158	2346-3′4′
159	2345-3′5′
Hexa-Chlorobiphenyls (Cont.)	
160	23456-3′
161	2346-3′5′
162	235-3′4′5′
163	2356-3′4′
164	236-3′4′5′
165	2356-3′5′
166	23456-4′
167	245-3′4′5′
168	246-3′4′5′
169	345-3′4′5′
Hepta-chlorobiphenyls	
170	2345-2′3′4′
171	2346-2′3′4′
172	2345-2′3′5′
173	23456-2′3′
174	2345-2′3′6′
175	2345-2′3′5′
176	2346-2′3′6′
177	2356-2′3′4′
178	2356-2′3′5′
179	2356-2′3′6′
180	2345-2′4′5′
181	23456-2′4′
182	2345-2′4′6′
183	2346-2′4′5′
184	2346-2′4′6′
185	23456-2′5′
186	23456-2′6′
187	2356-2′4′5′
188	2356-2′4′6′
189	2345-3′4′5′
190	23456-3′4′
191	2346-3′4′5′
192	23456-3′5′
193	2356-3′4′5′
Octa-chlorobiphenyls	
194	2345-2′3′4′5′
195	23456-2′3′4′
196	2345-2′3′4′6′
197	2346-2′3′4′6′
198	23456-2′3′5′
199	2345-2′3′5′6′
200	23456-2′3′6′
201	2346-2′3′5′6′
202	2356-2′3′5′6′
203	23456-2′4′5′
204	23456-2′4′6′
205	23456-3′4′5′
Nona-chlorobiphenyls	
206	23456-2′3′4′5′
207	23456-2′3′4′6′
208	23456-2′3′5′6′
Deca-chlorobiphenyl	
209	23456-2′3′4′5′6′

(Table from Johnson et al., 2006)

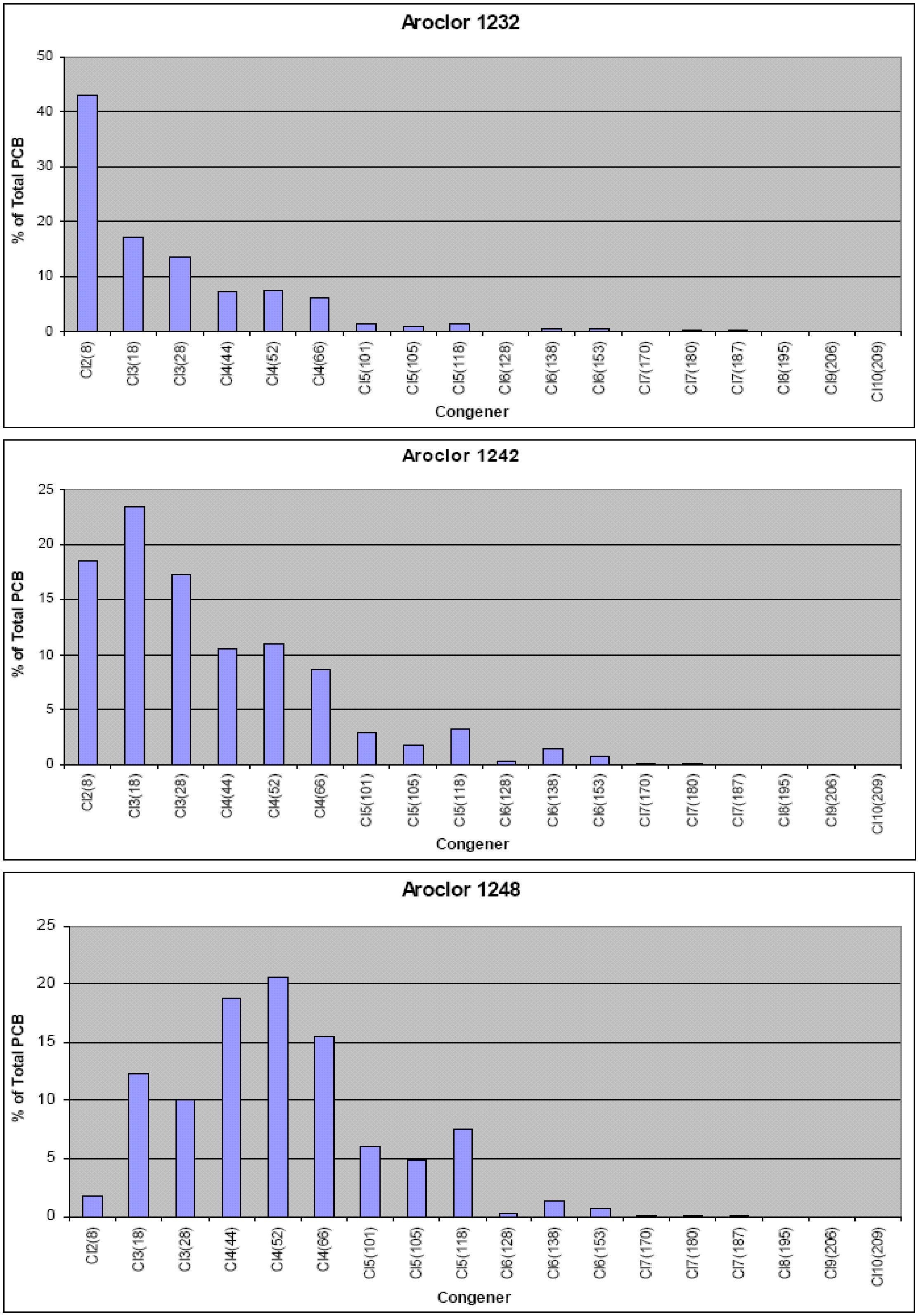


Figure 28. Aroclor 1232, 1242, 1248 compositions (example, source fingerprints).

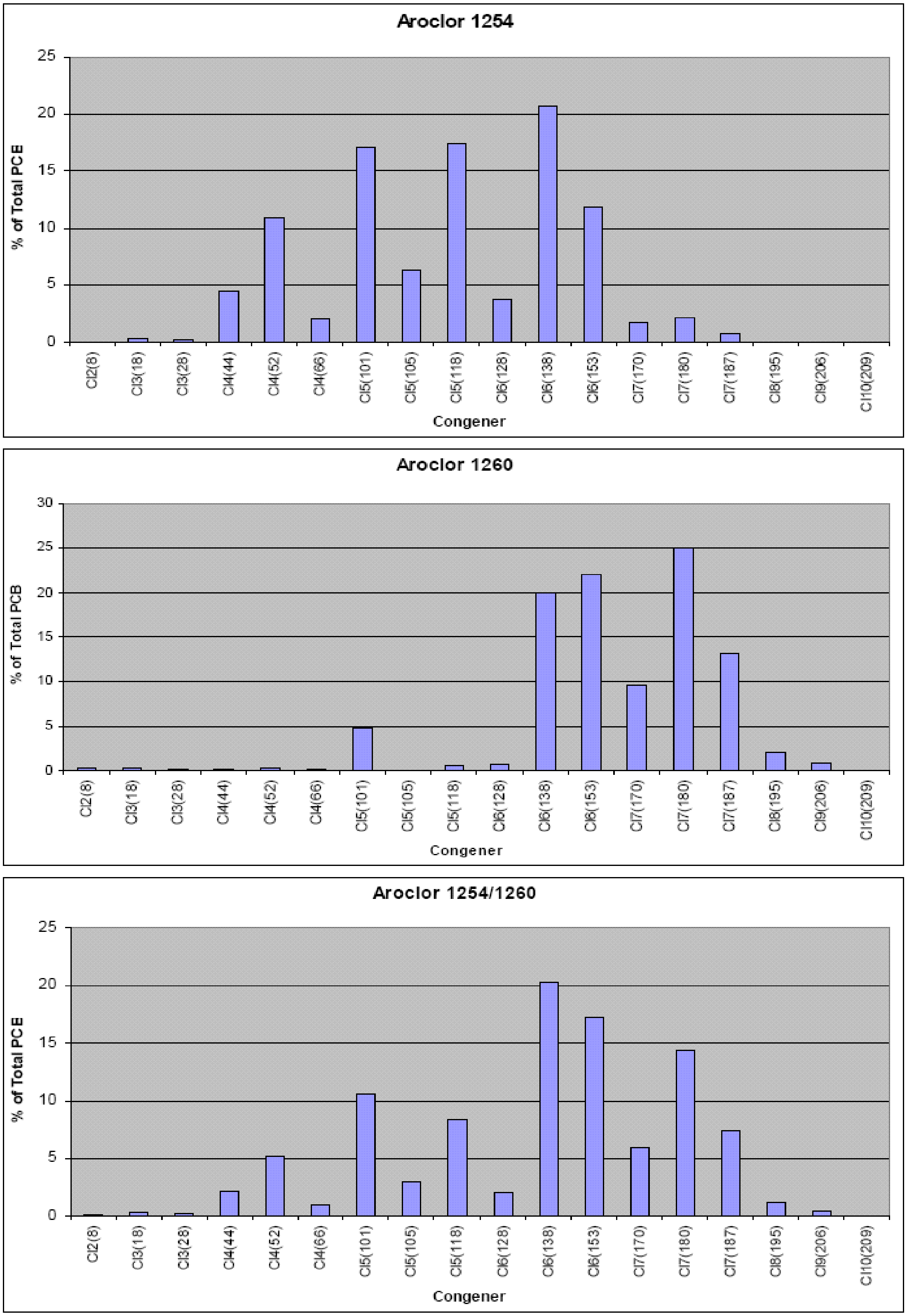


Figure 28 (cont.). Aroclor 1254 & 1260 compositions (source fingerprints).

The same properties that lead to their usefulness for industrial applications also lead to PCBs stability and persistence in the environment. PCBs possess a high molecular weight, which results in their desired high thermal stability, but also results in low water solubility and long lifetimes in the environment. Low solubility implies PCBs will tend to bind to solid soil and sediment particles rather than freely dissolved in water or volatilized to air. They possess high-octanol/water partitioning coefficients (k_{ow}) so they are lipophilic ("fat loving" rather than hydrophilic or "water loving") and tend to partition into organic phases. Their lipophilic nature means PCBs are fat-soluble and usually found associated with the total organic carbon (TOC) fractions in soils and sediments. When organisms consume PCBs they become associated with the lipid fraction of the organism, and are not easily metabolized or lost. These general PCB chemical properties result in PCBs biomagnifying, or increasing in concentration, as PCBs are consumed but not metabolized or lost from organisms in various food chains.

Although PCBs are persistent in the environment, they can still undergo degradation and alteration in the environment as well as within organism tissues. PCBs as a group are considered very stable and persistent, but they are actually a diverse mixture of congeners with varying chemical properties covering a wide range. Individual congeners are subject to degradation and alteration, which progresses at varying rates. Generally, the lighter (lower numbered in Table 5) congeners will be more soluble and volatile and therefore more susceptible to differential solubility and volatilization processes. Heavier congeners (higher numbered in Table 5) are more hydrophobic and tend to bioaccumulate up the food chain, and can be fractionated into the fatty tissues in organisms. Within organisms, different congeners will metabolize at different rates, so biological fractionation is still possible once PCBs are within the organism. Specific congeners are also the preferred target of microbial dechlorination in anaerobic settings, so such congeners can be altered by these processes as well. Taken together, all these potential alteration processes indicate that a "simple" exercise in fingerprinting a few PCB source signatures may become a much more difficult problem. It often requires knowledge of these potential alteration mechanisms to recognize the alteration effects before "seeing" the actual original source fingerprints. For example, compare Figure 29, which shows environmental samples (site sediments) to the Aroclor examples in Figure 28, including the mix of Aroclors 1254 and 1260 that best matches these environmental samples. But even visual inspection shows sediment samples with PCB153 as the most common congener in all samples, which does not directly match any Aroclor pattern, leading to the conclusion that some other alteration has occurred in the environmental samples so they do not exactly match the Aroclor source profiles.

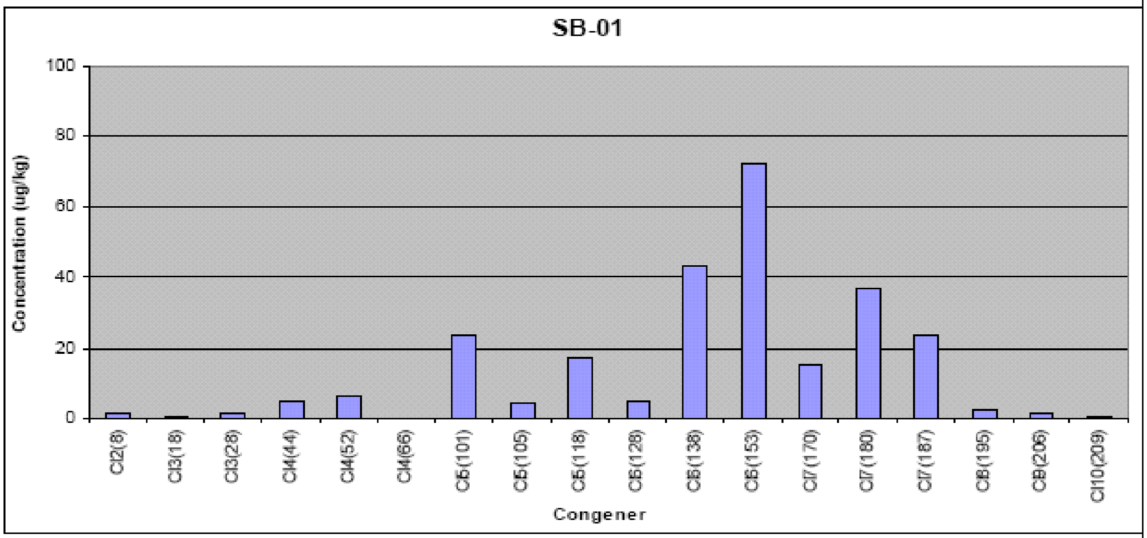
So as a result, although PCBs were produced in specific Aroclor mixtures of congeners, environmental samples usually contain congeners that are very different from those present in the original Aroclors. Once Aroclors are released into the environment, fate and transport processes lead to fractionation of their congener fingerprints. This will be discussed in the next section, in the context of limiting the use of Aroclor analyses to only fresh samples (for example, soils with freshly spilled PCB oils), whereas environmental samples typically require more advanced congener analyses. In aqueous settings, lower weight congeners are more easily solvated and can be transported away so remaining compositions become fractionated and possess a heavier mixture of congeners than that present in the original mixture. For example, an onshore spill of Aroclor oil results in a soil contaminated with PCBs with a congener mixture very similar to the original Aroclor. Subsequent erosion could bring soil particles into surface water bodies where many of the processes discussed above could result in PCB contaminated sediments with varying compositions. These sediments

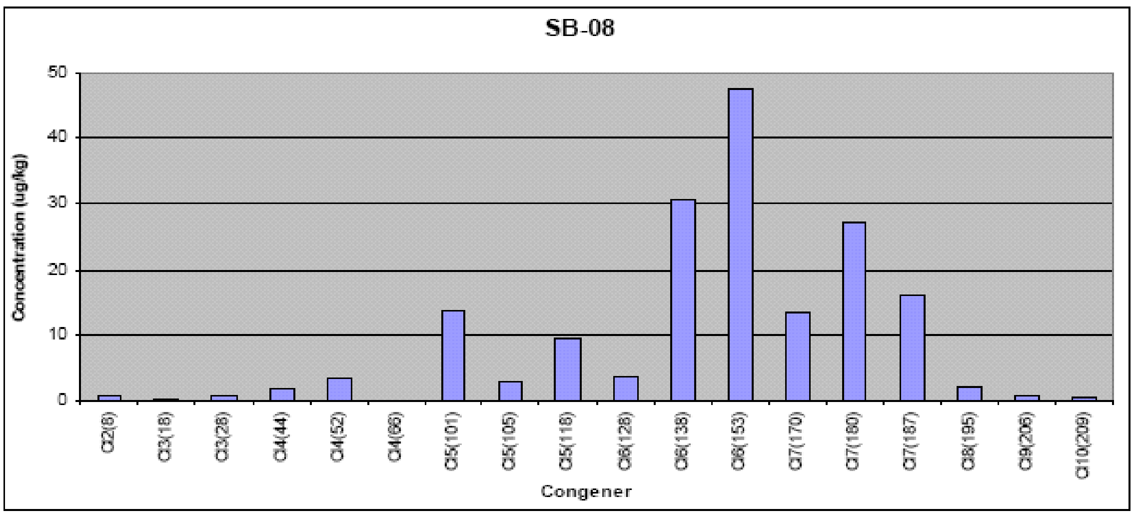
could have a very different PCB pattern then the original spilled Aroclor due to the loss of more soluble lower weight congeners through aqueous solubility processes that occur during transport from the original spill site to the deposited sediment location. Volatilization also occurs more readily in lighter congeners, as is demonstrated by air monitoring done at PCB remediation sites. Lab studies (Chiarenzelli et al., 1997) have shown significant volatile loss (>50% in lighter congeners) that is enhanced with repeated wetting and drying cycles, such as that seen in sediments on mudflats under tidal action. In anaerobic soil and sediments, certain bacterial groups dechlorinate PCBs. Long-term studies in contaminated sediments (Brown et al., 1987) have shown that specific bacterial groups show distinctive dechlorination patterns, transforming certain heavier congeners into lighter congeners as chlorines are removed. These dechlorination patterns have been used in fingerprinting studies to follow the changes in patterns to reconstruct the original source fingerprints (Magar et al., 2005).

Since PCBs are lipophilic and environmental exposures may be traced to ingestion of contaminated tissues, it is often desired to relate these tissue PCB patterns to potential source patterns. However, biological fractionation in PCB patterns can occur from both differential uptake and loss seen at various steps up a food chain. In this type of biological fractionation, some congeners may pass across cell membranes during uptake differentially compared to the other congeners. Likewise, during metabolism different congeners may show preferential losses, so again biological fractionation can occur. For these and perhaps other confounding factors, it becomes increasingly difficult to trace PCB patterns from tissues back to original sources. However, if the various sources display distinct enough patterns, and these types of alteration processes contribute only slight changes to these patterns, it is still possible to link PCB tissue data to sediment sources. Some studies (Glenn et al., 2006) are even looking at fingerprinting human blood samples to match ingested fish as likely exposure sources for PCBs in human health studies. Overall, this short review demonstrates the need to understand PCB fate and transport processes in the environment since they have effects on the observed PCB congener patterns seen in environmental matrices. If exposure pathways are going to be traced back to original sources, these types of physical, chemical and biological alteration processes must be better understood. Given all the limitations in our understanding of these alteration processes, this review will focus mainly on the problems associated with determining the sources of PCBs to the sediments, which, act as the major reservoir for PCBs in the aquatic environment.

5.3 PCB ANALYTICAL METHODS

Before choosing an analytical method, one must determine whether to measure total Aroclor, homolog groups, or individual congeners. To select which types of analytical methods are required for a forensics study, a decision must be made about how the data will be used. Will there be a need to measure individual congeners, and if so, exactly how many and which ones? Can the study goals be accomplished with a mix of methods that include some less expensive techniques that only provide homolog or total Aroclor data? A complete planning of the study objectives along with review of data quality objectives will lead to the selection of appropriate analytical methods. As was found with PAHs (Stout et al., 2003), many of the PCB analytical techniques developed for regulatory programs may not necessarily be appropriate for forensic studies. Many U.S. EPA methods were developed for regulatory programs (such as the Superfund or CERCLA program) and require strict adherence to procedures outlined in U.S. EPA's SW-846 Methods (EPA, 1997). However, the goals of many of these regulatory programs are to determine contaminant "nature and extent," which is not always sufficient for a forensics study with the goal to determine





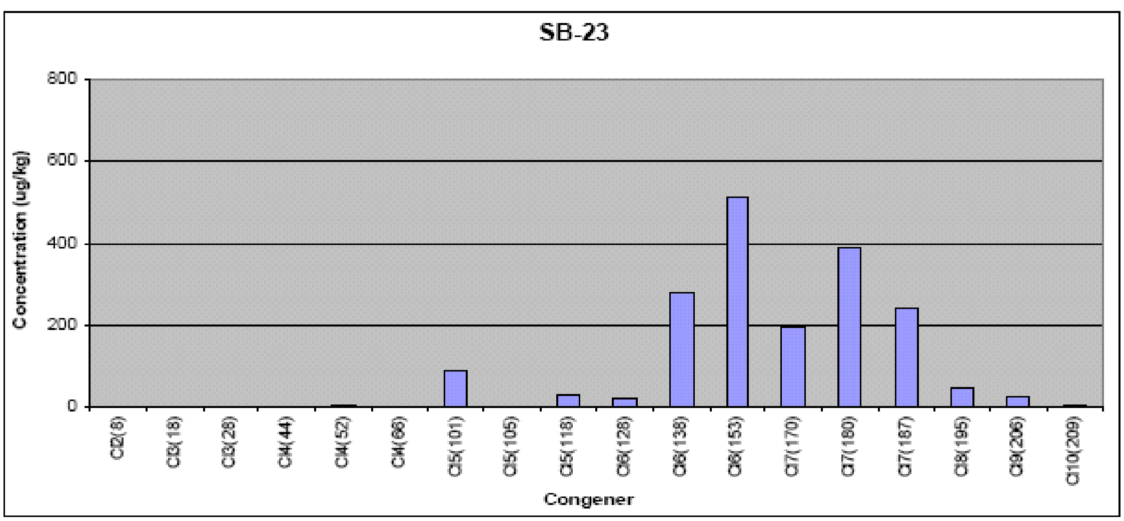


Figure 29. Sample PCB Compositions.

the sources of contamination. Forensic studies may require slight modifications in standard methods to obtain the appropriate data. EPA has recognized this and is moving more towards performance based measurement systems (PBMS) rather than strict adherence to SW-846 methods. In most cases, the types of analyses discussed here for forensic study will also meet these PBMS requirements, and with adequate planning, these types of data can be used for both forensic and regulatory purposes. Use of a mixture of various methods together may be needed to develop the most efficient analytical program to meet the defined goals of a well-developed forensics study.

Before looking at specific analytical methods, it is important to consider the sampling techniques and various sample matrices that are available. In the previous section, discussions noted that PCBs tend to be particle bound and found in the solid matrix. Even in studies that looked at PCBs in water samples (Johnson et al., 1997), the majority of the PCBs were associated with the unfiltered solids in the water samples. By looking at suspended material in water samples, it is possible that recent source information can be determined for identifying continuing sources that are the targets of compliance programs, e.g., Total Maximum Daily Loads (TMDLs). Surface sediments obtained with surface grabs provide comparable data on recent sources, and often provide the same information as the suspended material in water samples. Without some form of dating technique, it is difficult to determine whether suspended material in water samples represents material recently washed into the water body from onshore sources versus recycled bottom sediments re-suspended into the overlying water. Deeper sediment core samples (again with the aid of some form of dating techniques) may provide a historical record of source contributions to a water body. Depending on the temporal and spatial needs of the selected study design, a mix of sample matrices and sampling efforts may be required. Some forensics studies (such as the Delaware and Hudson River cases discussed in the Introduction) have sampled both sediment and fish tissue at the same locations to follow PCB pathways through the food chain. If fish consumption is considered a human health risk, it is useful to trace the source of PCBs before considering any potential remedial strategies. All of these potential sample types have significant implications for what types of sample analyses will be appropriate for the particular forensics study design. It is therefore critical to develop a conceptual site model to explain what is occurring at the site and plan a complete DQO process for sample collection and analysis before any of the of following analyses are attempted.

5.3.1 Immunoassay Methods

A simple and relatively inexpensive option for Total Aroclor data should include immunoassay methods. Recent advances in the environmental science have followed the medical field in the use of enzyme-linked immunosorbent assays (ELISA). These immunoassays include reaction steps for a competitive reaction between unknown sample PCBs and kit-provided PCB conjugates (PCBs with added color indicators that are activated in later reaction steps). Antibody sites where this competitive reaction occurs have traditionally been on the “frosted” sides of test tubes, but more recent advances have led to antibody sites on free-floating magnetic particles within the test tube solutions to provide better precision and accuracy. These ELISA methods can be employed in the field as near real-time “field analytics,” or in the laboratory with more control (for environmental factors such as temperature, etc.) and QC (additional replicates, duplicates, and standards as additional time permits) as desired. Under field or laboratory conditions, these immunoassay techniques are covered under U.S. EPA method 4035. For solid matrices, they generally consist of a field-capable extraction step to put the contaminant of interest into solution,

whereas samples already in solution can be run with virtually no time-consuming preparation. Contaminant concentrations are generally related to a color change that is either visually observed or quantified by spectrometer. Samples tend to be run in large batches (20-50 samples) along with a standard series of Aroclors for direct comparisons of concentrations. So although the immunoassay detects individual PCBs, individual PCB quantities are not determined and total quantities are reported in Aroclor equivalents relative to the standard Aroclor reference that was analyzed concurrently with the sample batch. In addition to immunoassays, further information on other rapid characterization methods can be found online at the U.S. EPA Clu-in website (http://clu-in.org/).

5.3.2 Total Aroclor Laboratory Methods

U.S. EPA methods 608 and 8082 are generally used to provide total Aroclor data for most liquid and solid matricies. Method 608 is the simplest laboratory method for analyses of waters using gas chromatography (GC) for separation and electron capture detection (ECD). GC columns are packed with specific materials that allow PCBs to move through at different rates, generally allowing lighter fractions to move faster and therefore exit or elute from the column first and be separated from the heavier PCBs that elute later. ECD provides rather unsophisticated detector capabilities, sensing any electro-negative constituents in solution, including chlorinated PCBs and pesticides. Simple ECD output consists of a chromatogram showing a series of peaks, with intensity in the y direction related to individual constituent concentration, and the time in the x direction representing the time for each constituent to pass through the GC column. (Again, earlier peaks represent lighter constituents, and later arriving constituents are represented by peaks out to the right on a standard chromatogram.) Few internal standards are employed, but use of multiple GC columns (packed with different materials to provide different elution times) is allowed during separation steps to provide for confirmation. Identification and quantification is made through comparisons to Aroclor standards that are run under the same conditions as the unknown samples. Method 8082 is slightly more involved and is generally employed for solid samples such as soils, sediments, and tissues. This laboratory technique uses a standard extraction method (such as Soxhlet or flow through) with clean-up steps to remove the contaminants from the solid matrix and put them into solution without other interfering constituents, a prerequisite for all methods other than Method 608 (which is limited to liquid samples where PCBs are already in solution). Method 8082 also uses GC/ECD (but can be modified with other detectors for congener analyses, see below section), but with more internal standards than used for method 608. With either method, as few as three to five peaks on the obtained chromatogram are compared to standard Aroclor peaks to determine which Aroclors are present, and quantities are determined based on extrapolation of Aroclor proportions of these few selected peaks. Since environmental samples tend to be altered from pure Aroclor mixtures, sometimes the Aroclor determinations and quantities are "best fits" to the peaks that are present in the chromatogram, which may no longer directly resemble fresh Aroclor mixtures. So comparisons to the more precise congener analyses listed below often result in total PCB levels that are higher or lower than expected, depending on the degree samples have been altered. These methods are therefore only recommended for "fresh" PCB samples. Direct measurement of PCB oils or water and soil with fresh spills might be appropriate candidates for these analyses. However, most environmental samples (water, soil, sediment, tissue) have had some alteration in the original Aroclor composition so it is really not appropriate to compare their compositions to fresh Aroclor patterns. It is likely the altered patterns in the environmental samples would require some "fitting" to a single or even mix of pure Aroclor

compositions, with the more altered samples requiring more guesswork to produce Aroclor concentrations. Generally, environmental samples should opt to have congener analyses done rather than strict Aroclor analyses. For this reason, forensic studies also often need to go the next level of analysis, and determine individual congener patterns to develop a fingerprint that can be used to discriminate various sources.

5.3.3 Congener Laboratory Methods

With certain modifications, some of the previous Aroclor methods can be adapted to provide additional congener data. With modifications including the use of Mass Spectrometry (MS) detectors, EPA method 8082 can provide a limited set of congener data (usually 20 to 30 congeners can be determined). MS detectors provide much more specificity than ECD, since mass is used to differentiate the various congeners. But this is often still insufficient for fingerprinting since although 20 peaks is better than the five peaks, alterations may still make original fingerprint patterns difficult to recognize. Method 680 is another GC/MS method and is used to determine homolog groups in water and solid samples. Although homolog groups can provide some insight into potential sources, any alterations such as those commonly seen in environmental samples will make determining potential source fingerprints confusing.

Prior to about 1997, forensic studies needed to live with the limitations of the above methods because that was all that was generally available at the time. U.S. EPA method 1668 uses high-resolution GC/MS and was developed to quantify the more toxic co-planar PCB congeners in water, soil, and sediment samples. In 1999, method 1668 was revised to U.S. EPA method 1668A, which allows for the determination of over 150 different individual congeners and is becoming the preferred method for forensics studies. When looking for so many congeners, the probability for individual congeners to co-elute (travel and exit the GC column at the same time) is higher, and even MS detectors may be unable to resolve individual congeners. Attempts have been made to run multiple GC columns with different retention times (columns packed with different materials) to enhance the separations, but results are still sometimes reported as combined totals for a number of congeners. The important point here is that the correct specific congeners should be quantified, rather than just a total of a group of congeners. It is important to identify the congeners that will provide the most information, to allow for the discrimination of the original sources or specific alteration processes in a forensics study. Ultimately, it may be more important to select a technique that identifies the critical congeners in the individual original sources, plus some congeners that evaluate alteration patterns so that those original patterns can be distinguished from other alteration processes that commonly affect environmental samples.

5.3.4 Selecting a Mix of Methods for Forensics Studies

Due to cost concerns, it is often preferable to select a mix of methods to meet the goals of a forensics study. Method 1668A is considered the ultimate method of choice for forensics studies with low to sub-pptr detection limits for each congener, but it can be costly, at greater than $1000 per sample. This compares to costs as low as $100 per sample for common total Aroclor analyses, which may only offer detection limits at 50 to 100 ppb. Immunoassays are commercially available at costs as low as $25 per sample, but detection limits may be higher than laboratory techniques and only total concentration information is available. Use of field-analytics such as immunoassays provide the benefit of mapping out gradients while still in the field and allow selection of a subset of samples that can also be run in the laboratory to provide congener specific data at very low detection limits.

It is often possible to still reach the goals of the forensics study at a lower cost by combining a number of methods in a well-planned study. A tiered study of this type was demonstrated for PAH (Stout et al., 2003) and PCB (Leather et al., in prep) forensics studies and serves as the basis for the following PCB forensic study design. By combining large numbers of inexpensive ($25 per sample) immunoassay samples with more expensive (200 to $1000 per sample) laboratory congener analyses, a cost-effective study design can be developed. A large number of low cost analyses allow sufficient spatial and temporal coverage to map out contaminant plumes and assist in locating multiple potential source areas. A subset of samples can then be selected for laboratory analyses to provide the distinctive congener fingerprints needed to match these sample areas to potential sources. This initial contour mapping provides an initial view of the site to better formulate a conceptual model that will allow better use of subsequent laboratory analyses. In this manner, expensive laboratory congener analyses are not wasted on non-detect samples that would not provide fingerprinting information. In a typical regulatory program, the “nature and extent” aspect of the project may delineate the general 3-D extent of the PCB contaminated plume. The objective of a forensics study might be to determine the various sources that contributed to this PCB plume. For a cleanup project, the overall goal of the forensics work might be to apportion remedial costs between various contributors. This requires adequate spatial coverage to delineate the boundaries of the PCB contamination and adequate understanding of the compositional variations within specific areas that are contaminated.

Good spatial coverage in heterogeneous media such as soil or sediment often requires large numbers of samples. As mentioned previously, the low cost and near real-time nature of immunoassays allow for a rapid and cost-effective method to delineate spatial boundaries. A subset of samples selected within these boundaries can then be sent for laboratory analyses to develop congener fingerprint patterns to determine the compositional variation within the plume. This might include an initial set of U.S. EPA method 8082 analyses for only the 18 NOAA Status and Trends congeners to obtain a basic understanding of the compositional variation around the site. This could be followed by samples for EPA method 1668A to provide more congeners at lower detection limits if required, e.g. if the 18 congeners from method 8082 were not distinct enough to discriminate among potential sources, due to either detection limit issues or alteration processes such as those discussed in Section 5.2.

For compliance issues such as TMDLs, a similar tiered approach could be employed. Core data can be used to show temporal variations in sources at a particular site. Again, the heterogeneous nature of the cores would likely require large numbers of samples to completely characterize the contributions from the various sources, so a tiered analysis program starting with relatively inexpensive immunoassays might be preferred. Once spatial and temporal variations in the concentration data are obtained, a subset of samples can be sent for laboratory analysis with confidence that minimal non-detect congener data will be obtained. For TMDL studies, it might also be necessary to search upstream in watersheds for any continuous sources, for which this same type of tiered approach can be followed. Since upstream sources should occur at higher concentrations with less alteration, a shift towards the lower cost analyses would also be advised. In this case, simple immunoassays moving upstream through the watershed might show concentration gradients leading to potential sources. These potential sources could subsequently quantified with laboratory techniques such as EPA method 8082. Variations of tiered programs could be developed depending on the needs of the project, so again the development of a conceptual site model and sufficient data quality objectives (DQO) would help determine the appropriate type of tiered effort .

Case studies will be developed in Section 5.6 to provide examples of some of these tiered forensic studies.

5.4 STUDY DESIGN FOR A PCB FINGERPRINTING PROJECT

The main goal of a forensics study is to look for similarities between the compositional makeup of sample chemistries and a set of very distinctive end-member source chemistries from which the sample fingerprint is composed. It helps to have a very distinct set of end-member sources that show some unique chemical properties so they can be easily discriminated from one another, and then mixed together in various proportions to provide the observed sample characteristics. If the compositional chemistries of the end-member sources are not very distinct and cannot be used to adequately separate samples into distinctive groups, then the forensics study may not be successful at determining distinct sources for the various samples. For PCBs, the data that are generally used are the mixture of congeners that constitute each sample. These sample congener patterns are then compared to a set of reference patterns, generally the original Aroclor compositions.

Unraveling the complexity of commingled or overlapping sources of PCB contamination in sediments near any facility requires good spatial (and perhaps temporal) coverage of the impacted sediments and a precise chemical characterization of the congener composition of the impacted sediments. These two requirements can be cost effectively achieved through the combination of (1) rapid sediment characterization (RSC) of a large number of sediments to identify contaminant trends, “hotspots,” and key samples using fast, semi-quantitative, and typically field-deployed methods and (2) subsequent Advanced Chemical Fingerprinting (ACF) of a selected subset of sediments to recognize and unravel distinct source “fingerprints” using more advanced laboratory and data analysis methods.

The objective of combining RSC with ACF is to cost effectively maximize the benefits of each method and to help offset the limitations of each method. For example, RSC provides a cost-effective technique for spatial (and perhaps temporal with core data) coverage, allowing chemical gradients to be determined for initial indications of potential sources. However, it only provides total Aroclor PCB data but does not allow individual congeners to be determined that are required for actually fingerprinting sources. ACF normally requires specialty analyses beyond the scope of normal regulatory requirements and beyond the capabilities of many commercial laboratories. For example, in the case of PCBs, many regulatory programs only require that the concentrations for total Aroclors or maybe 18 major congeners be determined and reported. However, ACF of PCBs requires that approximately 50 to 100 PCB congeners be determined at a higher analytical cost.

The combined use of RSC and ACF, however, are only two steps in the overall forensics process. The overall sequence of steps, or tasks, that should be employed include (1) evaluation of the site’s potential as a demonstration site, (2) development of a conceptual site model, (3) development of a defensible study design, (4) initial use of rapid sediment characterization (RSC) screening, (5) selection of a subset of samples for advanced chemical fingerprinting (ACF), and finally, (6) synthesis and presentation of the results in a final report. These steps are laid out in the following subsections of this section in more depth as an example of how one might plan and conduct a PCB fingerprinting study.

5.4.1 Step 1. Evaluating a Site's Potential for Forensic Study

The specific reason for whether and why a contaminant source study should be considered at a particular site will undoubtedly vary for each site. At some sites, the need will be obvious (e.g., the site owner is being held responsible for contamination for which they are not liable), whereas at others, the need will be less obvious (e.g., the site has agreed to clean up to 'background' levels, which are poorly established). Some considerations to help determine the need for a contaminant source study are discussed in the following subsections. The result of this first step is to determine whether or not the site would serve as a good candidate to utilize fingerprinting techniques for PCBs.

The most obvious and common consideration is whether or not it is possible non-site sources may have contributed to the known or suspected contamination near the site. This potential situation will be obvious in some locations where the site is surrounded by other industrial and commercial properties with long operational histories. This situation certainly favors that a contaminant source study be conducted to determine the potential contribution of the site to the 'total' contamination. Even at more isolated facilities, the potential exists for 'background' levels of contamination to rival or even exceed any reasonable site contribution to the sediments. In this situation, the site owner may be prudent to defensibly define the background (ambient) conditions (e.g., due to direct atmospheric fallout to a water body or natural 'background') and thereby limit their potential liability to only those areas where a site activity has impacted the water body above the background conditions.

Other considerations may include (1) what known or suspected contaminant PCB sources existed on the site property (now or in the past), (2) what known or suspected industries are (or were) located on nearby properties, (3) what are (or were) the known typical contaminants associated with those industries, (4) what are the general sediment transport dynamics of the area—i.e., could contamination get from "*here to there,*" and (5) how amenable will regulators and other stakeholders be to the use of such methods? Each question must be considered and weighed in determining if the site will serve as a good candidate to utilize PCB forensic techniques.

5.4.2 Step 2. Development of a Conceptual Site Model

Once a site's candidacy has been established, a conceptual site model (CSM) for the ensuing contaminant source study must be developed, or an existing CSM must be modified. For example, a CSM developed for the proposed site may already exist that can be augmented to include a preliminary synthesis of the contaminants, their candidate sources for the study area, and the potential for transport of sediments/contaminants. In many cases, a regulatory project for the site has already developed a CSM that can serve as a good starting point. At the completion of a CSM, it should be possible to accomplish the following: (1) identify (or confirm the identity) the known or suspected contaminants of potential concern (COPCs) for the site, (2) Identify all of the known or suspected sources of the COPCs within the study area, and (3) Develop specific objectives (hypotheses) to be evaluated by the contaminant source study that address the greatest environmental risks, and provide the greatest potential benefit, for the proposed forensics study site.

An important step in the identification or confirmation of contaminants of concern is a review of the pre-existing data for the study area (and nearby areas, which might provide additional insight to regional background issues). Pre-existing data may reside in published and unpublished sources. Published sources of data will reside primarily within the scientific

(journal) literature. Therefore, a library literature search of the study area could reveal published datasets related to earlier investigations by academic or industry researchers. In addition, inquiries to local universities may reveal that environmental studies have been conducted in the study area and unpublished data from master of science and doctoral theses may already exist. Other sources of unpublished data will include the data submitted to regional, state, or federal regulatory agencies by other groups (e.g., consultants to nearby industries) working within the study area. The primary problem with using pre-existing data for forensic study is that they normally represent different ‘vintages’ of data, collected at different points in time, and using different analytical methods and different laboratories. Each factor tends to introduce variables that limit the comparability and any comprehensive interpretation of the pre-existing data as a whole. Furthermore, the pre-existing data need to be reviewed with a very critical eye with respect to the data quality. For example, vintage data may suffer from inadequate analytical methods or, as is commonly the case, contain significantly elevated detection limits. Thus, the utility of the assembled data need to be carefully evaluated and interpreted within the context of when and how the data were collected.

Because of these shortcomings, pre-existing data typically only represent a starting point in providing defensible interpretations surrounding the source(s) of contamination within the study area. Assembled pre-existing data should be reviewed to help confirm the contaminants of potential concern (COPCs), potentially identify trends and hotspots, potentially identify candidate sources, and generally guide the study design of the contaminant source study. These existing data may be leveraged into Step 3, and provide cost savings when developing a defensible study design for a PCB forensics study.

5.4.3 Step 3 Development of Defensible Sampling Strategy

The development of a technically defensible sampling strategy requires a balance between meeting project objectives and data quality objectives within the budget of the project. The design is typically based on some type of statistically based sampling (e.g., random, systematic, stratified, cluster, etc.) and professional judgment based upon the information assembled in the CSM during Step 2 above. Sampling designs are often site-specific and require consideration of many aspects of the study design. These types of considerations are addressed in many outside references (e.g., Gilbert (1987), and references herein).

Professional judgment is a valuable tool in the study sampling design because it allows for site-specific knowledge to be incorporated into the design. For example, larger numbers of samples can be placed in the vicinity of known or suspected contaminant sources (e.g., NPDES, stormwater, marinas, or combined sewer overflow (CSO) outfalls), or in locations where historic releases are documented to have occurred, and fewer (or no) samples can be placed in areas where little sediment deposition is occurring or where dredging was recently completed. The climatic conditions may also be important to consider. For example, particulate loading to surface sediments near outfalls may be highest in the time following heavy rains or snow melt.

As part of developing this strategy, note how well-represented are the potential contaminant sources. In many instances, upland sampling of non-Navy properties will not be permitted. In the case of stormwater run-off, access to sediments within a catchment basin may simply require a permit from the city. However, in the case of a ‘hostile’ neighbor, access may be impossible and the sampling design strategy will require sampling in

sediments proximal to the inaccessible properties, usually at some point below the mean high water line. Thus, the sampling strategy needs to include consideration of how the legal issues balance with the best means of representing potential contamination from an inaccessible area.

The extent and density of sampling (i.e., spatial coverage) is usually the issue that requires the greatest consideration in developing a sampling design strategy. It is the number of samples that will largely determine the cost of the project. By using a tiered study design that allows RSC data to first contour concentrations, it can be designed to more cost-effectively use the ACF data. If it was determined that an objective of the study was to access historical inputs to the sediments, then the sampling design must include collecting at least some sediment cores that are intended to capture "pre-anthropogenic" contributions to the sediments. Such cores need to be placed in areas shown to be (or are believed to be) areas of sedimentary deposition and that have not been dredged. Radiogenic dating of core segments using radiogenic dating techniques (e.g., ^{210}Pb or ^{137}Cs) can yield sediments from particular 'time intervals' whose chemistry reflects conditions from those periods. This dating can be important in areas where historic (and now defunct) operations are considered to have been a significant source of the contamination to the study area.

Whatever the number of samples determined available for RSC is determined to be, some of these need to be reserved as "samples of opportunity" to be determined in the course of field work. Such samples could include any samples related to interesting or peculiar observations made in the course of the fieldwork. In addition, most contaminant source study objectives will necessarily include some assessment of the ambient (background) conditions within the study area. Thus, careful consideration must be given to where representative background samples can be obtained, including areas beyond the immediate study area. Given the importance of background samples in demonstrating the concentrations of contaminants beyond the control of the site, the number of background samples needed to meet the objectives of the study should be carefully considered. Population statistics are vital to the defensibility of the conclusions and should be qualitatively and quantitatively considered.

The final sampling design will be recorded in the forensics work plan that includes the locations of each sample to be collected for RSC (note the locations of the samples for ACF will not be determined until after the RSC data are acquired and interpreted). The project work plan should include maps of the sample locations and GIS coordinates, which will expedite their location during the sampling event. Producing maps of the planned sample locations before the field operation provides an opportunity to visually assess (and modify if necessary) the adequacy of the spatial coverage of the sampling design to meet the project objectives.

5.4.4 Step 4 Rapid Sediment Characterization (RSC)

Numerous RSC methods have been described in existing Navy guidance for other purposes (http://web.ead.anl.gov/ecorisk/issue/pdf/rsc.pdf), so the discussion here is brief. RSC of semi-volatile organics can be conducted using various immunoassay techniques. The techniques for the RSC of PCBs in sediments have been adapted from methods developed for use in soils (EPA Method 4020). They require dewatering of the sediment to below about 30% moisture by placing on filter paper to remove excess water. Sample preparation can be more involved and similar to standard laboratory methods, depending on the objectives of the project. For many applications, the more basic preparation methods that will still meet the

project needs and data quality objectives are usually selected so this initial step in the procedure can be conducted in a timely manner. The dewatered sediment is then extracted using solvent (e.g., methanol) and analysis of the extract is then conducted by ELISA (Enzyme-linked Immunosorbent Assay) methods. In summary, the extract is treated with specific antibodies that promote a color change depending upon PCB concentration, which is measured against a PCB standard solution-calibrated spectrophotometer.

Finally, the data are subjected to geostatistical treatment where concentration variations with distance are plotted (variograms) and used to generate contour plots of the data (see for example, Barabas et al., 2001). RSC data interpretation can significantly benefit from additional physical properties data for the sediments, if available. For example in the case of PCBs, these additional properties may include grain size or total organic carbon (TOC). Normalization of PCB data by these parameters often shows a background trend in the PCB data that can be separated from additional PCB sources. Grain size information also may assist in interpretation of sediment transport, which will lead to understanding of contaminant transport from sources to sediment sample locations. With additional sediment transport information, these chemical gradients (PCBs sorbed on sediments generally move from high concentration source areas to lower concentration depositional zones) can be used to suggest various PCB source areas. These contour maps (both surface and subsurface) display chemical gradients that indicate potential sources, with the 3-D aspect only being defined due to the number of low cost RSC samples that can be measured. These spatial presentations of the data show gradients and allow different source areas to be proposed for validation by the more detailed laboratory analysis (ACF). The benefit of using a tiered approach (using RSC to select ACF samples) is a cost-effective study design in a heterogeneous matrix such as sediments. If only higher cost forensic samples are measured, fewer locations are sampled and might miss potential sources due to heterogeneity.

Regardless of the approach used in the evaluation of RSC data, it is important to remember that the goal of the RSC data analysis is to develop a sufficient set of visual displays to aid in the selection of samples for ACF (and not to alone achieve the objectives of the study). The analytical strategy and budget will largely determine the number (or percentage of the RSC samples) that will be selected for ACF. Of course, it is not necessary that all of the ACF budget be used if there is no technical basis to do so. For example, if the RSC data have demonstrated an overwhelming consistency and predominance of 'background' ambient conditions in the study area, the ACF may simply include a few selected confirmation samples. Therefore, the task of selecting samples for ACF is largely a matter of selecting a reasonable and justified subset from the complete set of RSC samples. Some guiding principles to remember and keep in mind in the selection of samples for ACF are as follows:

1. Select samples that provide ample spatial coverage of the entire study area (try to represent all areas of the study and do not completely ignore any area on the basis of RSC alone).
2. Select a sufficient number of samples from specific location(s) within the study area that address a specific project objective(s) (i.e., select sufficient samples in areas of specific concern or interest, potentially including accessible upland sites of interest).

3. Select samples that represent the range of RSC concentrations observed, including those that are (apparently) representative of the ambient/background conditions (i.e., do not exclude all the low concentration samples as they may provide important information on "background" conditions).

Of course, an underlying basis for the selection of samples for ACF to meet these guidelines is the cost. Thus, a degree of professional judgment is still needed in the selection of samples for ACF.

5.4.5 Step 5. Advanced Chemical Analysis (ACF)

The need for ACF methodology rests with the limitations of standard EPA methods (SW-846) to meet the objectives of a contaminant source study (Stout et al., 2003). The fundamental shortcoming with virtually every conventional EPA SW-846 method of analysis, when used for measuring contaminants, particularly organic contaminants in sediments and other media, is a lack of detailed measurements of those *diagnostic* chemicals known to comprise these complex mixtures. Instead, these methods are focused on selected compounds identified as "priority pollutants," which are quite pervasive in contaminant mixtures (e.g., different petroleum products) and generally insufficient to distinguish different sources of otherwise similar contaminants (Douglas and Uhler, 1993). Because of these limitations, chemists at some environmental laboratories have modified the standard EPA methods to yield the data necessary to support detailed contaminant source investigations. With respect to these modified methods, note that the EPA SW-846 guidelines allow flexibility in the deployment of the 'standard' analytical methods. While most commercial laboratories are not interested in modifying the standard methods, some laboratories have the experience and flexibility to modify standard methods to meet project goals without violating the standard method guidelines. When properly planned, most data generated by ACF methods can support contaminant source studies and convention regulatory assessment programs. In other words, the ACF data can generally be considered defensible and accepted by regulatory agencies if the data quality objectives are clearly defined and met by the effort.

The ACF techniques available for the assessment of semi-volatile organic contaminants in sediments (e.g., PCBs) are all based upon high-resolution gas chromatography, usually operated in conjunction with compound-specific detectors (e.g., ECD or MS). Battelle has in recent years developed state-of-the-art PCB analytical methods using high-resolution gas chromatography/low-resolution mass spectrometry operating in selected ion monitoring mode (HRGC/LRM-SIM), that are both highly cost effective and provide detailed, high-quality data (Durell, 2001; Durell and Seavey, 2000). The method employs components of EPA Method 680 (HRGC/LRMS PCB homologue and total PCB method) and Method 1668a (HRGC/HRMS PCB congener method). The base methods were modified to include a large number of non-standard environmentally important and diagnostic PCB congeners that will permit data analysis for differentiating potential sources.

Once a subset of samples has been selected for ACF, a forensic analysis for PCBs will include the characterization of more than 100 discrete PCB congeners (congeners that comprise >98% of the total and possible PCB contamination; Attachment 1c), which enables scientists to apply a variety of powerful data interpretation methods. PCB forensics data reduction and analysis include various types of statistical and other numerical analyses, forensics graphing/plotting/mapping, cross plotting, cluster and principal component analysis for similarity and dissimilarity analysis, analysis for determining the age of the

contamination, and determination of degradation and dechlorination activity etc. More detailed descriptions of these forensics methods, including specifically for PCBs, have been presented and documented elsewhere (including in Jarman et al., 1997; Johnson et al., 2000; Durell et al., 2001; Durell and Higman, 2001; Emsbo-Mattingly and Durell, 2003; Magar et al., 2005; Johnson et al., 2007; and Leather et al., in prep).

5.4.6 Step 6. Data Interpretation and Reporting

The manner by which the results and conclusions of a contaminant source study are conveyed needs to consider the audience, particularly whether they are technical or lay decision-makers. Naturally, the specific target audience will dictate the level of technical detail conveyed in a report or presentation. Chemical 'fingerprinting' data in graphical and/or tabulate form can be very confusing to all but an experienced chemist. Their interpretation is much easier (and thereby useful) when the results of a contaminant source study are reported using numerous visual demonstratives that either convey the data spatially or some other easily interpreted visual (e.g., contour maps such as those shown in Appendix D). Such visuals can be more readily explained to and interpreted by technical and non-technical audiences. This is important since the value of any contaminant source study will be undermined if the audience cannot understand the results and conclusions. Some of these presentation techniques for RSC and ACF have been discussed in Steps 4 and 5 above.

Once the data from samples and potential end-member sources have been collected, they are generally put into a database in preparation for data analysis. Data are often viewed in matrix form with sample locations along one axis and individual congeners listed along the other axis. The first step, even before data analysis, is to determine whether some data issues may arise in ensuring data are of comparable quality. If too many samples contain data below required detection limits, replacement with detection limit values or two times the detection limits (a common practice with lower quality data) may result in artifacts when latter multivariate statistical analyses are run. A general rule of thumb is to not replace more than 10% of the non-detect data with some inserted values. It might be better to drop those samples that show large numbers of non-detect data, or at least remove those congeners that show non-detect data from the latter analyses. If data are from various sources (different labs, different techniques, different collection or run dates, etc.), subtle differences may also show up as artifacts in latter statistical analyses. These differences could be interpreted as representing different sources when it only reflects the different types of data. Therefore, ensuring data are all of the same quality is important, and also checking back latter if any observed trends in the data are related to these types of data differences rather than source differences.

Another topic that merits discussion prior to data analysis is regarding data normalization. Data are usually obtained from the lab in units of concentration, such as micrograms per kilogram or parts per billion. These absolute concentration levels are important for determining concentration gradients, where higher levels tend to be found closer to the source of the contamination. With greater distances from the source, total concentrations are diluted to lower levels but proportions remain consistent (assumes no preferential alteration processes discussed in previous section have fractionated various congeners). So, to trace the constant proportions of the distinct sources among all the samples with varying concentrations, environmental scientists often use some form of data normalization. The most common technique is to divide each congener concentration by the total concentration of the sample, so units are viewed as percentage or fraction of total

concentration. If no normalization is done, concentration differences overwhelm the ability of most latter statistical analyses from grouping samples with similar compositional variations.

In the simplest cases, visual inspection of the data may be sufficient to discern the mix of end-member compositions that will result in the sample chemistries. However, in most cases, some form of multivariate analysis must be used to look for trends in the data and suggest end-member source contributions that have been mixed together to produce the individual sample chemistries. The types of multivariate analyses that are available for investigation of forensics datasets include hierarchical cluster analysis (HCA), principal component analysis (PCA), and polytopic vector analysis (PVA). These are not the only techniques that are available, but are some of the more common ones found in many forensic studies, so they will be discussed here.

5.4.6.1 HCA (Hierarchical Cluster Analysis)

In HCA, distances between pairs of samples (or other variables) in a dataset are calculated and compared using agglomerative clustering algorithms. When distances between samples are relatively small, this implies that the samples are similar, at least with respect to PCB distributions. When distances between samples are relatively large, this implies that the samples are dissimilar. HCA can be performed on either samples or variables (PCB congeners). Clustering of samples reveals similarities among the samples, while clustering of variables pinpoints inter-relationships between variables. As such, HCA is an additional useful data analysis and exploration tool.

5.4.6.2 PCA (Principal Component Analysis)

Exploratory data analysis tools such as PCA algorithms are designed to reduce large and complex data sets to a reduced set of combined variables, or principal components. PCA can be used to explore the variability among the PCB composition in the samples. Specifically, the outputs of PCA are two- or three-dimensional factor score plots in which the principal component scores for each sample is cross-plotted. If a significant portion of the variance in the dataset is accommodated in the first few principal components (PCs), then the Euclidean distances between sample points on such plots (e.g., PC1 v PC2 or PC2 v PC3) provide a clear measure of their chemical similarity. Samples that visually “cluster” are chemically similar and vice versa. Another form of PCA output, factor-loading plots, can also be used to determine which individual variables (in our case PCB congeners) are responsible for any visual “clustering” observed. As such, PCA is a useful data analysis and exploration tool. An example of a PCA plot is shown in Figure 32, with samples showing a trend in compositions between Aroclors 1260 and 1254. This plot could support an interpretation of two end-member sources, one from the Navy with an Aroclor 1260 pattern and one from the creek with a mixture of 1260 and 1254. This type of plot might be used to assign responsibility between these two sources depending on where samples fall on this trend line between the two potential sources.

5.4.6.3 PVA (Polytopic Vector Analysis)

Receptor modeling such as PVA requires a large amount of experience and should therefore follow established methodologies such as those outlined by Johnson, et al. (2007). The data will be carefully reviewed to assess the impact of low concentration samples, non-detects, and the presence of outliers. The appropriate data-screening action is project-specific and may include (1) data correction/normalization, (2) removal of samples from the data set, and (3) removal of congeners/peaks from the data set. After the data are prepared as outlined

above, the resultant data matrix should be analyzed using a multivariate receptor modeling method such as polytopic vector analysis (PVA). The first step in this process is the determination of the number of fingerprints in the system. Criteria used to determine the number of fingerprints (or sources) include the normalized loadings method, the signal-to-noise criterion, and inspection of goodness-of-fit indices and scatter-plots as described by Johnson (2007). The next step in the receptor modeling process is to resolve the EM compositions (source profiles) and mixing proportions (source contributions) within each sample. In PVA, this involves use of the DENEG algorithm of Full et al. (1981). The algorithm iterates until all the samples meet a defined non-negative convergence criterion. The final step in the process is to (1) compare the resolved end-member congener profiles with known or suspected source patterns (i.e., Aroclor) and alteration mechanisms (e.g., literature reported dechlorination methods – Bedard and Quensen, 1995) and (2) map of the end-member mixing proportions both temporally and geographically.

5.5 TECHNOLOGY GAPS

Recent advances in isotope ratio GC/MS have allowed the use of isotopic variations to assist in fingerprinting PCBs. The use of this technique began in the mid 1990's with carbon isotopic variations in PAHs (as discussed in Stout et al., 2003), and PCB isotopic studies soon followed (Jarman et al., 1998). One of the most important advances in technology has been compound specific isotopic analysis (CSIA) to allow each individual congener to be analyzed separately, rather than mixing all the various congeners together into a bulk isotopic signal. So for carbon isotopic analysis the GC is used to separate the various congener compounds, then each is combusted separately to form carbon dioxide gas that is analyzed by isotope ratio MS. For forensic studies this allows selection of specific congeners that are more resistant to alteration to be used for analysis to avoid changes in source patterns that might be related to alteration rather than original source signatures. This is important, for example, since many bacterial processes favor the use of lighter isotopes (where bonds are easier to break) and result in slight isotopic fractionations that leave the remaining carbon pool with heavier carbon isotopes. By looking at only those congeners that show little alteration changes in the source signatures, it is easier to perform forensic studies. Although the CSIA techniques show great promise, continued work is required to lower detection limits and reduce interference from coeluting peaks for various congeners.

5.6 CASE STUDIES

PCB forensic studies have been done in many areas, including the Great Lakes, Hudson River, Narraganset Bay, Lake Hartwell, and others. The Navy has also performed some preliminary fingerprinting for PCBs along the south shore of Hunters Point Shipyard (HPS). The following is a brief summary of those HPS studies with more discussion available as a full case study in Appendix D.

HPS is a former Navy installation located on a peninsula in the southeast corner of San Francisco, California (Figure 30). HPS comprises about 955 acres, with approximately 457 acres of offshore sediment (Parcel F). From 1945 to 1974, the Navy maintained and repaired ships at HPS. The facility was deactivated in 1974 and remained relatively unused until 1976, when it was leased to Triple A Machine Shop, a private ship repair company. In 1986, the Navy resumed occupancy of HPS. The facility was closed in 1991 under the Defense Base Realignment and Closure Act of 1990 (BRAC) and is in the process of conversion to non-military use. The South Basin area is a shallow embayment on the south side of HPS, with water depths ranging from 6 ft to less than 2 ft. No streams or rivers enter South Basin except

for Yosemite Creek, a shallow, tidally influenced channel with no permanent flow. Prior to 1965, three CSOs discharged to this area: one at the head of Yosemite Creek, one on the north side of the creek near Griffith Street, and one on the south side near Fitch Street. All wet weather overflows were directed to the CSO at the head of Yosemite Creek after 1965. Contaminants identified during investigations of Yosemite Creek by the City and County of San Francisco (CCSF) included PCBs, polycyclic aromatic hydrocarbons (PAHs), pesticides and metals.

Historical activities in adjacent upland Parcel E-2 (Figure 30) that may have contributed to contamination of sediments in South Basin include filling and disposal activities, residual onshore contamination, and surface runoff. Groundwater discharge was also evaluated as a potential transport pathway of PCBs to South Basin from Parcel E-2, however, the magnitude of PCB release via this pathway is not likely to be significant given the limited extent of PCBs detected in groundwater and their low solubility. A former landfill at Site IR-01/21 in Parcel E-2 (Figure 30) was used from 1958 to 1974 for the disposal of materials such as construction and industrial debris and waste, domestic refuse, sandblast waste, paint sludge, solvents, waste oils, transformers and electrical equipment and other potentially contaminating materials. No records that document landfill contents or disposal practices are available. In the mid-1970s, the Navy placed 2 feet of compacted imported fill on top of the landfill and graded the entire site to facilitate storm water drainage. In the 1990s, a sheet pile wall was installed and riprap was placed along the Parcel E-2 shoreline to control the movement of contaminants into South Basin. In 2001, an interim landfill cap was constructed and placed over most of the landfill. The cap consists of a multilayer system of sub-base soil, HDPE membrane, synthetic drainage layer, and topsoil.

In Figure 31, RSC screening data in the South Basin from an earlier regulatory study have been mapped to show two distinct areas of higher concentrations. One higher concentration area to the northeast adjacent to the landfill in Parcel E-2 and a lower one at the mouth of Yosemite Creek to the west are suggested by the gradients in the data. Based on these data along with the previous site history information, a conceptual site model could be developed for this site. The ACF techniques could then be employed to validate that these are distinct sources and also apportion these two sources in the surrounding sediments. Figure 32 contains a principal component analysis (PCA) plot from a HPS South Basin sediment area as an example of how ACF data might be presented. Samples that plot close together in principal component space have similar chemical composition, and on this plot site, samples from existing studies that analyzed for 18 congeners display a trend from an Aroclor 1260 composition (lower left in plot) up to a 50% mixture of Aroclors 1260 and 1254 (upper right center in plot). Unfortunately, with only 18 congeners any dechlorination that may be occurring with depth (and aging of the sediment horizons) cannot be differentiated from this simple mixing trend. It is therefore important to look at a greater number of congeners (including those congeners specific to particular dechlorination pathways) to discriminate between source mixing such as that indicated in this PCA plot and any dechlorination that may be occurring with depth. The benefit of using a tiered approach (using RSC to select ACF samples) is a cost-effective study design in a heterogeneous matrix such as sediments. If only higher cost forensic samples are measured, fewer locations are sampled and might miss potential sources due to heterogeneity.

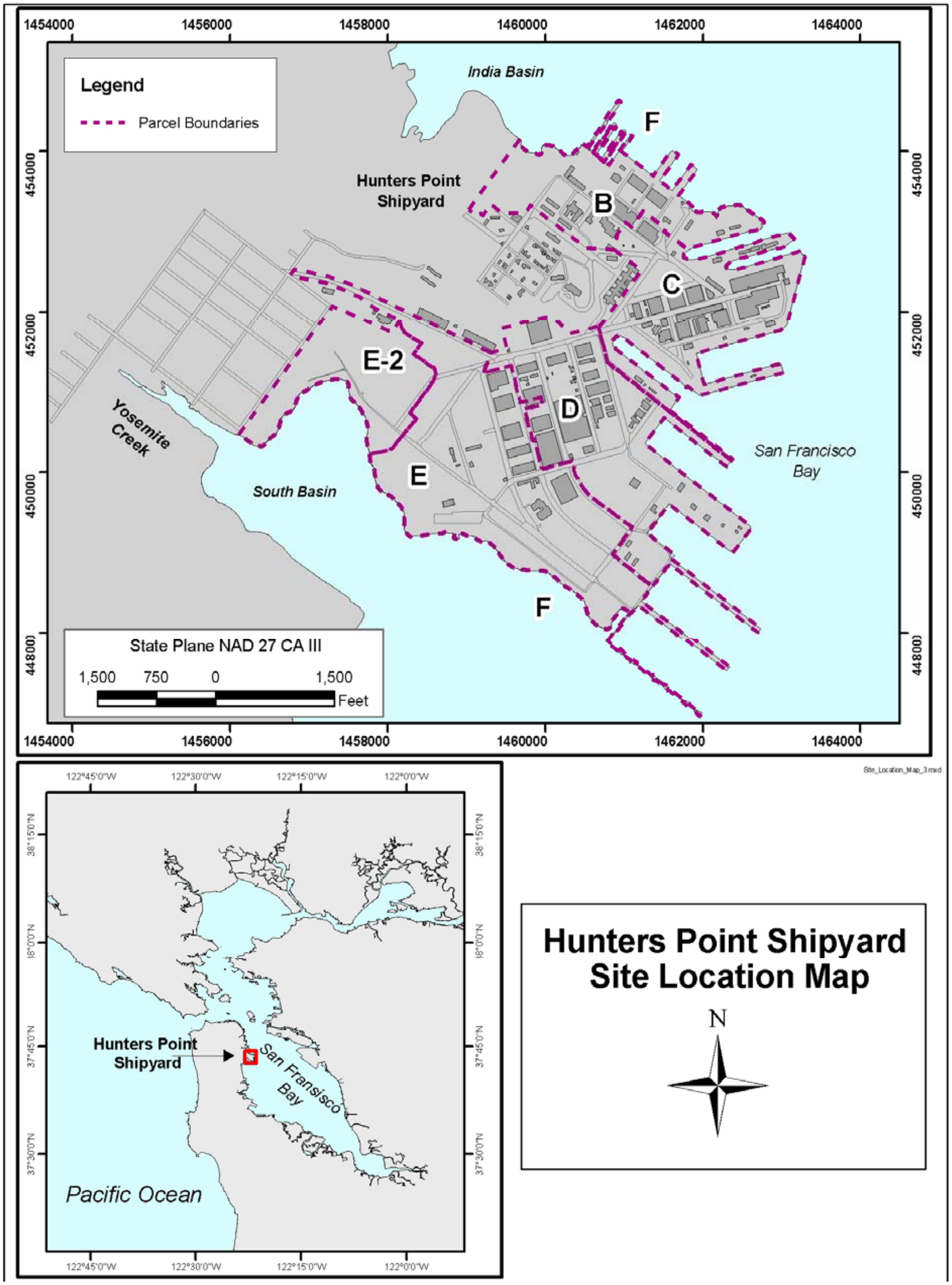


Figure 30. HPS site location map.

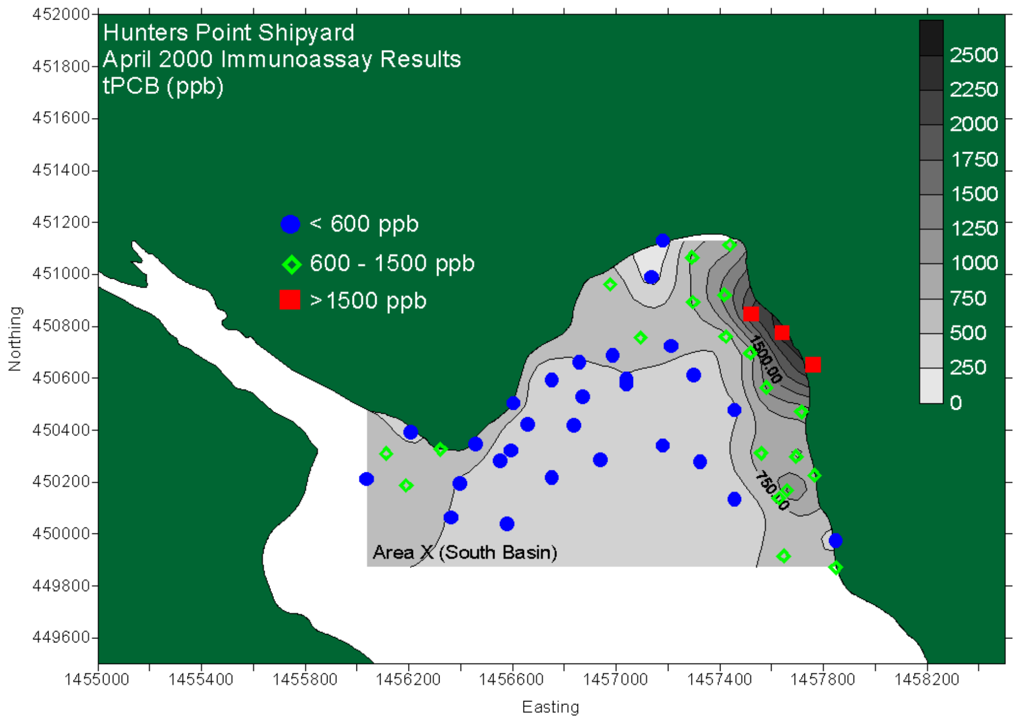


Figure 31. PCB surface contour map of South Basin sediment area.

In Figure 32, first (PC1) and second (PC2) principal components show samples plotted between compositions representing Aroclor 1260 (bottom left) and Aroclor 1254 (upper right). Samples near Navy site plot closer to Aroclor 1260 composition and those samples closer to City Creek plot closer to a mixture of Aroclor 1260 and 1254.

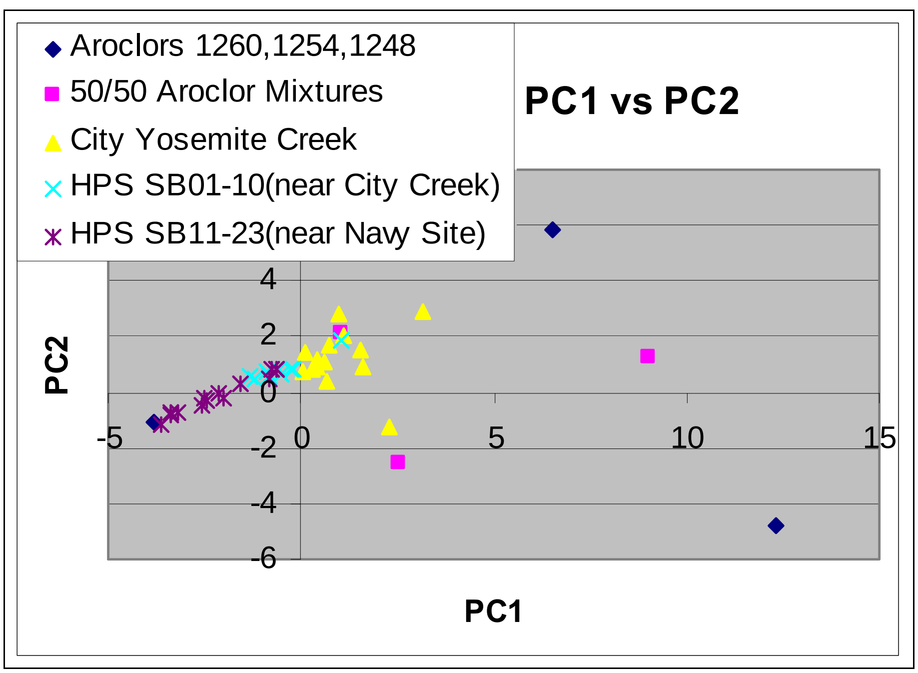


Figure 32. Principal Component Analysis (PCA) plot.

5.7 RELATIONSHIP TO OTHER ORGANIC CONTAMINANT FORENSIC STUDIES

The suggested procedure for the PCB forensics approach outlined here follows closely from the earlier forensic review for PAHs (Stout et al., 2003) that showed how this procedure could be applied in the Elizabeth River area around Norfolk Naval Shipyard. This approach is used for the PCB forensics effort that is currently being performed as part of ESTCP Project ER-0826 (Leather et al., in prep). This same approach could also be used with pesticides with only minor variations. The laboratory analytical techniques are generally the same, as these techniques provide data on both PCBs and pesticides. The generated data matrix would have various pesticide constituents rather than PCBs quantified as input to multivariate data analysis techniques.

5.8 CONCLUSIONS

The overall forensics study design should use a combination of inexpensive, more general analyses at the start for establishing concentration gradients followed by more expensive, specific congener analyses for actual multivariate fingerprinting techniques. This might include a tiered analytical program that starts with immunoassay or lower cost laboratory analysis to map out concentration gradients to build an initial conceptual site model. The concentration gradients can help suggest possible source areas to guide the forensics study in the selection of samples for additional laboratory analysis. Several different laboratory analyses are available for forensic studies depending on the cost, detection limit, and number of congeners that are required by the particular study. In most cases, the congener data are arranged in a matrix and some form of multivariate analysis is used to compare the site data to potential source patterns to determine the number of sources and ultimately the percent contributions of each potential source to each site sample.

6. REFERENCES

References are currently in draft form, and divided by section chapters above.

Introduction

California State Water Resources Control Board (SWRCB). 2003. State of California S.B. 469 TMDL Guidance: A Process for Addressing Impaired Waters In California.

NAVFAC. 2006. TMDL Prioritization Report.

U.S. EPA (draft). 1999. Draft Guidance for Water Quality-based Decisions: The TMDL Process (Second Edition), EPA 841-D-99-001. http://www.epa.gov/owowwtr1/tmdl

Microbial Source Tracking:

Bruce A. Wiggins, Philip W. Cash, Wes S. Creamer, Scott E. Dart, Preston P. Garcia, Todd M. Gerecke, Jennifer Han, Brian L. Henry, Kylie B. Hoover, Erica L. Johnson, K.C. Jones, Jacquie G. McCarthy, Justin A. McDonough, Sarah A. Mercer, Michael J. Noto, Haewon Park, Matthew S. Phillips, Stephanie M. Purner, Brian M. Smith, Erin N. Stevens, and Amy K. Varner. 2003. "Use of Antibiotic Resistance Analysis for Representativeness Testing of Multiwatershed Libraries." *Applied and Environmental Microbiology* 69(6), 3399–3405.

Cole, D., S.C. Long and M.D. Sobsey.: "Evaluation of F+ RNA and DNA Coliphages as Source-Specific Indicators of Fecal Contamination in Surface Waters. Appl. Environ. Microbiol. Dev. 69, 6507–6514 (2003).

EPA, U.S. National Beach Guidance and Required Performance Criteria for Grants. Office of Science and Technology within EPA's Office of Water. **EPA-823-B-02-004** , 1–23. 2002a.

EPA, U.S. National Beach Guidance and Required Performance Criteria for Grants. Office of Science and Technology within EPA's Office of Water. **EPA-823-B-02-004** , 1–23. 2002b.

EPA, U.S. Microbial Source Tracking Guide Document. Office of Research and Development. EPA/600-R-05-064, 53–54. 2005. Cincinnati, OH, US EPA.

EPA, U.S. Water: Monitoring and Assessment. Chapter 5.11 Fecal Bacteria. 2001.

G.M.Brion, J.S. Meschke, and M.D. Sobsey. F-specific RNA coliphages: occurrence, types, and survival in natural waters. Water Research 36, 2419–2425. 2001.

Griffin,D.W., R.Stokes, J.B. Rose and J.H. Paul III: Bacterial Indicator Occurrence and the Use of an F+ Specific RNA Coliphage Assay to Identify Fecal Sources in Homosassa Springs, Florida. Microbial Ecology Dev. 39, 56–64 (2004).

Ioana G.Petrisor, Rachel A. Parkinson, Jacqui Horswell, James A.Water, Leigh A.Burgonyne, David E.A. Catcheside, Winnie Dejonghe, Natalie Leys, Karolien Vanbroekhoven, Priyabrata Pattnaik and Duane Graves: Microbial Forensics. In: Environmental Forensics, eds. Robert D. Morrison and Brian L. Murphy (American Press, San Diego) pp. 227–257 (2006).

J.L.Mancini. Numerical estimates of coliform mortality rates under various conditions. Water Poll. Control Fed. 50, 2477–2484. 1978.

Jayson D. Wilbur and John E. Whitlock: Statistical Issues in Microbial Source Identification. In: Microbial Source Tracking, eds. Jorge W.Santo Domingo and Michael J. Sadowsky (ASM Press, Washington, DC) pp. 181–210 (2007).

Jill R. Stewart, Jan Vinjé, Sjon J.G. Oudejans, Geoff I.Scott, and Mark D. Sobsey. Sequence variation among group III F-Specific RNA coliphages from water samples and swine lagoons. Applied and Environmental Microbiology 72(2), 1226–1230. 2006.

Jin, G., H. W. Jeng, H. Bradford, and A. J.Englande. Water Environ Research 76(3), 245-255. 2004.

Johnson,L.K., M.B. Brown, E.A. Carruthers, J.A. Ferguson, P.E. Dombek and M.J. Sadowsky: Sample Size, Library Composition, and Genotypic Diversity among Natural Populations of Escherichia coli from Different Animals Influence Accuracy of Determining Sources of Fecal Pollution. Appl.Environ.Microbiol. Dev. 70, 4478–4485 (2004).

Joyce Simpson, Jorge W. Santo Domingo, and Donald J. Reasoner. Microbial Source Tracking: State of the Science. Environmental Science & Technology 36(24), 5279–5288. 2007.

Kathy Ellis. Understanding Clean Water Act. Currents: The Navy's Environmental Magazine Summer, 48–49. 2002. Defense Environmental Network & Information Exchange (DENIX) Website.

Lim, G.E. and M.G. Haygood: "Candidatus Endobugula glebosa," a Specific Bacterial Symbiont of the Marine Bryozoan Bugula simplex. Appl.Environ.Microbiol. Dev. 70, 4921–4929 (2004).

Maiden, M.C., J.A. Bygraves, E. Feil, G. Morelli, J.E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D.A. Caugant, I.M. Feavers, M. Achtman and B.G. Spratt: Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenicámicroorganisms. PNAS Dev. 95, 3140–3145 (1998).

Marilyn Soule, Edward Kuhn, Frank Loge, John Gay, and Douglas R. Call. Using DNA Microarrays To Identify Library-Independent Markers for Bacterial Source Tracking. Applied and Environmental Microbiology 72(3), 1843–1851. 2006.

Miller,W.G., M.D. Englen, S. Kathariou, I.V. Wesley, G. Wang, L. Pittenger-Alley, R.M. Siletz, W. Muraoka, P.J. Fedorka-Cray and R.E. Mandrell: Identification of host-associated alleles by multilocus sequence typing of Campylobacter coli strains from food animals. Microbiology Dev. 152, 245–255 (2006).

Moore,D.F., V.J. Harwood, D.M. Ferguson, J. Lukasik, P. Hannah, M. Getrich and M. Brownell: Evaluation of antibiotic resistance analysis and ribotyping for identification of faecal pollution sources in an urban watershed. Journal of Applied Microbiology Dev. 99, 618–628 (2005).

NAVFAC. Navy TMDL Assessment Phase II – Prioritization Assessment. 2003. Naval Facilities. Engineering Command. Prepared for Chief of Naval Operations (CNO) N451E.

Ron Gauthier, Dr. Robert George, Heather Halkola, Christine In, Dr. Jim Leather, Dr. Paulette Murphy, Dr. Ken Richter, Peter Seligman, Gerry Key, Scott Steinert, and Aldis Valkirs. Navy TMDL Technical Guidance. Ron Gauthier. 2006. NAVFAC. (GENERIC) Ref Type: Report

Salina Parveen, Kenneth M. Portier, Kevin Robinson, Lee Edmiston, and Mark L.Tamplin. Discriminant Analysis of Ribotype Profiles of *Escherichia coli* for Differentiating Human and Nonhuman Sources of Fecal Pollution. Applied and Environmental Microbiology 65(7), 3142–3147. 1999a. 12-15-2006a.

Salina Parveen, Kenneth M. Portier, Kevin Robinson, Lee Edmiston, and Mark L. Tamplin. Discriminant Analysis of Ribotype Profiles of *Escherichia coli* for Differentiating Human and Nonhuman Sources of Fecal Pollution. Applied and Environmental Microbiology 65(7), 3142–3147. 1999b. 12-15-2006b.

Scott, T.M., S. Parveen, K.M. Portier, J.B. Rose, M.L. Tamplin, S.R. Farrah, A. Koo and J. Lukasik: Geographical variation in ribotype profiles of Escherichia coli isolates from humans, swine, poultry, beef, and dairy cattle in Florida. Appl.Environ.Microbiol. Dev. 69, 1089–1092 (2003).

Soule, M., E. Kuhn, F. Loge, J. Gay and D.R. Call: Using DNA Microarrays To Identify Library-Independent Markers for Bacterial Source Tracking. Appl.Environ.Microbiol. Dev. 72, 1843–1851 (2006).

Thakur, S., W.E. Morrow, J.A. Funk, P.B. Bahnson and W.A. Gebreyes: Molecular epidemiologic investigation of Campylobacter coli in swine production systems, using multilocus sequence typing. Appl.Environ.Microbiol. Dev. 72, 5666–5669 (2006).

U.S. EPA: Water quality standards handbook (US Environmental Protection Agency, Office of Water, Washington, DC) (1994).

Wiggins, B.A., P.W. Cash, W.S. Creamer, S.E. Dart, P.P. Garcia, T.M. Gerecke, J. Han, B.L. Henry, K.B. Hoover, E.L. Johnson, K.C. Jones, J.G. McCarthy, J.A. McDonough, S.A. Mercer, M.J. Noto, H. Park, M.S. Phillips, S.M. Purner, B.M. Smith, E.N.Stevens and A.K.Varner: Use of Antibiotic Resistance Analysis for Representativeness Testing of Multiwatershed Libraries. Appl.Environ.Microbiol. Dev. 69, 3399–3405 (2003).

Wilbur, J.D. and J. E. Whitlock. 2007. Statistical Issues in Microbial Source Identification, p. 181–210. *In* Jorge W.Santo Domingo and Michael J.Sadowsky (eds.), Microbial Source Tracking. ASM Press, Washington, DC.

Yishu Song, Nari Talaty, W.Andy Tao, Zhengzheng Pan, and R.Graham Cooks. Rapid ambient mass spectrometric profiling of intact, untreated bacteria using desorption electrospray ionization. Chemical Communication , 61–63. 2007.

Metals Source Tracking:

Bermin, J., D. Vance, C. Archer, and P.J. Statham. 2006. The determination of the isotopic composition of Cu and Zn in seawater. Chemical Geology 226:280–297.

Bloom, N. S., and E. A. Crecelius. 1984. Determination of silver in sea water by coprecipitation with cobalt pyrrolidinedithiocarbamate and Zeeman graphite-furnace atomic absorption spectrometry. Analytica Chimica Acta **156**: 139–145.

Bruland, K.W., G.A. Knauer, and J.H. Martin. 1978. Cadmium in northeast Pacific waters. *Limnology & Oceanography*, **23**: 618–625.

Bruland, K.W. 1980. Oceanographic distribution of cadmium, nickel and copper in the North Pacific. *Earth Planetary and Science Letters*, 47: 176–198.

Bruland, K.W., K.H. Coale, and L. Mart. 1985. Analysis of seawater for dissolved cadmium, copper and lead – an intercomparison of voltammetric and atomic-absorption methods. *Marine Chemistry* **17**: 285–300.

Callahan, M.R., E.A. Kaltenbacher, and R.H. Byrne. 2004. In-Situ measurements of Cu in an estuarine environment using a portable spectrophotometric analysis system. Environmental Science & Technology 38(2): 587–593.

Chadwick, D.B., A. Zirino, I. Rivera-Duarte, C.N. Katz, and A.C. Blake. 2004. Modeling the mass balance and fate of copper in San Diego Bay. Limnology & Oceanography 49(2): 355–366.

Chadwick, D.B., I. Rivera-Duarte, A. Carlson, G. Rosen, L. Kear-Padilla, A. Zirino, T. Boyd, J. Gieskes, and O. Holm-Hansen. 2005. Determining the Fate and Ecological Effects of Copper and Zinc Loading in Estuarine Environments: A Multi-Disciplinary Program. , Report to the Strategic Environmental Research and Development Program, Project CP-1156, December 2005, 101 pages.

Chadwick, D.B., and M.H. Salazar. 1991. Integrated measurement technologies for monitoring the marine environment. In: Proceedings of Oceans 91 Meeting, October 1-3, 1991, Oceanic Engineering Society of the Institute of Electrical and Electronics Engineers, Honolulu, Hawaii, pp: 343–350.

Chapman, J.B., T.F.D. Mason, D.J. Weiss, B.J. Coles, and J.J. Wilkinson. 2006. Chemical separation and isotopic variations of Cu and Zn from five geological reference materials. Geostandards and Geoanalytical Research 30:5–16.

Cheng, R.T., V. Casulli, and J.W. Gartner. 1993. Tidal, Residual, Intertidal Mudflat (TRIM) model and its applications to San Francisco Bay, California. *Estuarine, Coastal and Shelf Science* **36**:235–280.

Chester, R. 1990. *Marine Geochemistry*. Unwin Hyman, Ltd., London, UK, p. 698.

Cottingham, K. 2004." ICPMS: It's Elemental." *Analytical Chemistry* 76(1): 35A– 38A (Product Review).

Di Toro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P.R. Paquin, and R.C. Santore. 2001. Biotic ligand model of the acute toxicity of metals, 1. Technical basis. *Environmental Toxicology and Chemistry* **20**(10): 2383–2396.

Earley, P.J., G. Rosen, I. Rivera-Duarte, R. Gauthier, Y. Arias-Thode, J. Thompson, and B. Swope. 2007. A Comprehensive Copper Compliance Strategy: Implementing Regulatory Guidance at Pearl Harbor Naval Shipyard & Intermediate Maintenance Facility. Technical Report 1952, SPAWAR Systems Center San Diego.

Esser, B.K., and A. Volpe. 2002. "At-sea High resolution Trace Element Mapping: San Diego Bay and its Plume in the Adjacent Coastal Ocean," 36(13): 2826–2832. *Environmental Science & Technology.*

Felton, M.J. 2003. "Plasma Opens New Doors In Isotope Ratio MS." *Analytical Chemistry* **75**(5): 119A–123A.

Flegal, A.R., T.F. Duda, and S. Niemeyer. 1989. "High Gradients Of Lead Isotopic Composition In North-East Pacific Upwelling Filaments," *Nature* **339**(6224): 458–460.

Flegal, A.R., G.J. Smith, G.G Gill, S. Sañudo-Wilhelmy, and L.C.D. Anderson. 1991. "Dissolved Trace Element Cycles in the San Francisco Bay Estuary," *Marine Chemistry* **36**: 329–363.

Gledhill, M., M. Nimmo, S.J. Hill, and M.T. Brown. 1997. "The Toxicity Of Copper(II) Species to Marine Algae, with Particular Reference to macroalgae," *Journal of Phycology* **33**: 2–11.

Johnson, H.D., J.G. Grovhoug, and A.O. Valkirs, 1998. Copper Loading to U.S. Navy Harbors. SSC San Diego Technical Document 3052, San Diego, CA.

Johnson, T.M. 2004. "A Review Of Mass-Dependent Fractionation of Selenium Isotopes and Implications for Other Heavy Stable Isotopes," *Chemical Geology* 204:201–214.

Katz, C.N. and D.B. Chadwick. 1993. "Synoptic Mapping And Modeling Of A Sewage Outfall Plume." In *Proceedings of the Marine Technology Society 1993 Conference, Marine Technology Society*, Long Beach, CA, pp: 207–215.

Manning, T.J. and W.R. Grow. 1997. "Inductively Coupled Plasma – Atomic Emission Spectrometry," *The Chemical Educator* 2(1): 1–19.

Marechal, C.N., P. Telouk, and F. Albarede. 1999. "Precise Analysis of Copper and zinc Isotopic Compositions by Plasma-source Mass Spectrometry," *Chemical Geology* 156:251–273.

Markl, G., Y. Lahaye, and G. Schwinn. 2006. "Copper Isotopes as Monitors of Redox Processes In Hydrothermal Mineralization," *Geochimica et Cosmochimica Acta* 70:4215–4228.

Montlucon, D. and S.A. Sañudo-Wilhelmy. 2001. Influence of Net Groundwater Discharge o the Chemical Composition of a Coastal Environment: Flanders Bay, Long Island, New York," *Environmental Science & Technology* 35(3): 480–486.

Naval Facilities Engineering Command. 2003. TMDL Phase 1 Screening Assessment Report prepared for the Chief of Naval Operations N45E1).

Naval Facilities Engineering Command. 2006. Navy Total Maximum Daily Load Prioritization Report, September 2006

Kura, B., and R. Tadimalla. 1999. “Characterization of Shipyard Wastewater Streams.” In M.A. Champ, T.J. Fox and A.J. Mearns (Eds.). *Proceedings, Oceans ’99, Special Session on Treatment of Regulated Discharges from Shipyards and Drydocks*. Seattle, Washington, September 13-16, 1999, pp: 17–25.

Olmez, I., E.R. Sholkovitz, D. Hermann, and R.P. Eganhouse. 1991. “Rare Earth Elements in Sediments Off Southern California: A New Anthropogenic Indicator,” *Environmental Science & Technology* **25**(2): 310–316.

Orians, K.J. and E.A. Boyle. 1993. The Determination of Picomolar Concentrations of Titanium, Gallium, and Indium in Seawater by Inductively Coupled Plasma Mass Spectrometry (ICPMS) following an 8-Hydroxyquinoline Chelating Resin Preconcentration,” *Analytica Chimica Acta* **282**:63–74.

PRC. 1997. Report of Copper Loading to San Diego Bay, California, Report to California Regional Water Quality Control Board, San Diego Region, 14 May 1997.

Santore, R.C., D.M. Di Toro, P.R. Paquin, H.E. Allen, and J.S. Meyer. 2001. “Biotic Ligand Model of the Acute Toxicity of Metals. 2. Application to Acute Copper Toxicity in Freshwater Fish and Daphnia. *Environmental Toxicology and Chemistry* **20**(10): 2397–2402.

Sañudo-Wilhelmy, S.A. and A.R. Flegal. 1991. “Trace Element Distributions in Coastal Waters Along the US-Mexican Boundary: Relative Contributions of Natural Processes vs. Anthropogenic Inputs,” *Marine Chemistry* **33**: 371–392.

Sañudo-Wilhelmy, S.A. and A.R. Flegal. 1992. “Anthropogenic Silver in the Southern California Bight: A New Tracer of Sewage in Coastal Waters,” *Environmental Science & Technology* **26**(11): 2147–2151.

Schiff, K. and D. Diehl. 2002. “Assessment of Trace Metal Emissions from Antifouling Paints during Underwater Cleaning Events and Passive Leaching. International Congress on Marine Corrosion and Fouling,” San Diego, CA, 21–26 July 2002.

Seligman, P.F., A.O. Valkirs, J.S. Caso, I. Rivera-Duarte, and E. Haslbeck. 2001. “Copper Release Rates from Antifouling Marine Coatings and their Relationship to Loading and Toxicity in San Diego Bay, California. In *Symposium on Pollution Prevention from Ships and Shipyards*. Oceanology International 2001 Conference, Miami, Fla., April 4-5, 2001. M.A. Champ, Ed. pp. 64–81.

Shafer, M.M., D.E. Armstrong, J. Hemming, and D. Tang. 2005. Speciation, Sources, and Bioavailability of Copper and Zinc in DoD-Impacted Harbors and Estuaries. Report to the Strategic Environmental Research & Development Program, Project CP-1158, February 2005, 121 pages.

U.S. EPA. 1992. Method 200.13. Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption. Revision 1.0, November 1992. http://www.epa.gov/waterscience/methods/method/files/200_13.pdf

U.S. EPA. 1993. Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Metals Criteria. Memorandum from Acting Assistant Administrator for Water M.G. Protho. U.S. Environmental Protection Agency, Office of Water, Washington, DC, Document 822/F-93-009.

U.S. EPA. 1993. Guidance Document on Dissolved Criteria: Expression of Aquatic Life Criteria, October 1993.

U.S. EPA. 1994. Method 200.7. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry. Revision 4.4, EMMC Version, http://www.epa.gov/waterscience/methods/method/files/200_7.pdf

U.S. EPA. 1994. Method 200.8. Determination of Trace Elements In Waters And Wastes By Inductively Coupled Plasma - Mass Spectrometry. Revision 5.4, EMMC Version, http://www.epa.gov/waterscience/methods/method/files/200_8.pdf

U.S. EPA. 1994. Method 200.9. Determination of Trace Elements by Stabilized Temperature Graphite Furnace Atomic Absorption. Revision 2.2, EMMC Version, http://www.epa.gov/waterscience/methods/method/files/200_9.pdf

U.S. EPA. 1996. Method 1669. Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. U.S. Environmental Protection Agency, Office of Water, Washington, DC. http://www.epa.gov/waterscience/methods/method/inorganics/1669.pdf

U.S. EPA. 1997. Method 1640. Determination of Trace Elements In Water by Preconcentration and Inductively Coupled Plasma-Mass Spectrometry. April 1997, http://www.epa.gov/waterscience/methods/method/files/1640.pdf

U.S. EPA. 1997. Method 200.12. Determination of Trace Elements In Marine Waters By Stabilized Temperature Graphite Furnace Atomic Absorption. Revision 1.0, September 1997.

U.S. EPA. 1998. Proposed Phase I Uniform National Discharge Standards for Vessels of the Armed Forces. Technical Development Document. U. S. Environmental Protection Agency, Office of Water, Washington, DC, Document EPA 821-R-98-009.

US EPA draft, 1999. Draft Guidance for Water Quality-based Decisions: The TMDL Process (Second Edition), EPA 841-D-99-001. http://www.epa.gov/owowwtr1/tmdl

U.S. EPA. 2003. The Biotic Ligand Model: Technical Support Document for Its Application to the Evaluation of Water Quality Criteria for Copper. U.S. Environmental Protection Agency, Office of Water, Washington, DC, Document EPA 822R03027, November, 2003.

Wang, P.F., D.B. Chadwick, I. Rivera-Duarte, and M. Brand. 2008. "Modeling Fate and Transport of Copper Species in San Diego Bay using TRIM2D." Manuscript in preparation.

Weiss, D., E.A. Boyle, V. Chavagnac, M. Herwegh, and J. Wu. 2000. "Determination of Lead Isotope Ratios in Seawater by Quadrapole Inductively Coupled Plasma Mass Spectrometry after Mg(OH)2 Coprecipitation," *Spectrochimica Acta B* **55**: 363–364.

Wu, J. and E.A. Boyle. 1997. "Low Blank Preconcentration Technique for the Determination of Lead, Copper and Cadmium in Small-volume Seawater Samples by Isotope Dilution ICPMS," *Analytical Chemistry* **69**:2464–2470.

Zhu, X.K., et al. 2002. "Mass Fractionation Processes of Transition Metal Isotopes," *Earth and Planetary Science Letters* **200**:47–62.

Zhu, X.K., R.K. O'Nions, Y. Guo, N.S. Belshaw, and D. Rickard. 2000. "Determination of Natural Cu-isotope Variation by Plasma-source Mass Spectrometry: Implications for Use as Geochemical Tracers," *Chemical Geology* **163**:139–149.

Organic Source Tracking:

Barabas, N.; P. Goovaerts, and P. Adriaens. 2001."Geostatistical Asssessment and Validation of Uncertainty for Three-Dimensional Dioxin Data from Sediments in an Estuarine River," *Environ. Sci. Technol* **35**: 3294–3301.

Barabas, N.; P. Goovaerts, and P. Adriaens. 2004. "Modified Polytopic Vector Analysis to Identify and Quantify a Dioxin Dechlorination Signature in Sediments. 2. Application to the Passaic River," *Environ. Sci. Technol* **38**: 1821–1827.

Bedard, D.L. and J.F. Quensen. 1995. "Microbial Reductive Dechlorination of Polychlorinated Biphenyls." In *Microbial Transformation and Degradation of Toxic Organic Chemicals;* L.G. Young and C.E. Cerniglia, Eds., pp. 127–216. Wiley-Liss, New York, N.Y.

Brown, J.F., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan, and R.J. May, 1987. "Environmental Dechlorination of PCBs," *Environ. Toxicol. Chem.* **6**:579–593.

Bzdusek, P.A., J. Lu, and E.R. Christensen. 2006. "PCB Congeners and Dechlorination in Sediments of Sheboygan River, Wisconsin, Determined by Matrix Factorization Environ," *Sci. Technol.* **40**: 120–129

Bzdusek, P.A., E.R. Christensen, C.M. Lee, U. Pakdeesusuk, and D.L. Freedman, 2006. "PCB Congeners and Dechlorination in Sediments of Lake Hartwell, South Carolina, Determined from Cores Collected in 1987 and 1998," *Environ. Sci. Technol*. **40**:109–119.

Chiarenzelli, J., R. Scrudato, and M. Wunderlich, 1997. "Volatile Loss of PCB Aroclors from Subaqueous sand," *Environ. Sci. Technol.* **31**:597–602.

Douglas, G.S. and A.D. Uhler. 1993. "Optimizing EPA Methods for Petroleum-Contaminated Site Assessments," *Environ. Test. Anal.* **2**:46–53.

Durell, G.S. and J. Higman. 2001. "Systematic Assessment of the Relevance of Sediment Contamination in a Florida River Basin." 22nd SETAC Meeting, November 11–15, Baltimore, MD.

Durell, G.S., V. Magar, and R. Brenner. 2001. "Natural Dechlorination and Detoxification of PCBs in a Contaminated Sediment Environment. International Conference on Remediation of Contaminated Sediments." Venice, Italy, October 10–12, 2001.

Durell, G.S. 2001. Identification of Contaminants of Concern: Remediation of in the Cedar and Ortega River. Final Report submitted to Camp Dresser & McKee, Inc., Jacksonville, FL, and the St. Johns River Water Management District, Palatka, FL. Battelle, Duxbury, MA.

Durell, G.S. and J. Seavey Fredriksson. 2000. "PCB Congener and PCB Homologue Analysis by HRGC/LRMS; A Low MDL, Cost Effective Analytical Alternative for Tomorrow's Quality Sensitive PCB Assessments." 23rd EPA Conference on Analysis of Pollutants in the Environment, May 14–16, Pittsburgh, PA.

Emsbo-Mattingly, S. and G. Durell. 2003. "Identifying PCB Mixtures and Compositional Changes in Sediments with Low-resolution Mass Spectrometry and Environmental Forensic Interpretation Tools." *Seventh International In Situ and On-site Bioremediation Symposium*," June 2-5, Orlando, FL.

Full, W. E., R. Ehrlich, and E. Klovan. 1981. "Extended Q models Objective Definition of External End Members in the Analysis of Mixtures," *J. Math. Geol*. **13**: 331–344.

Gilbert, R. O. 1987. *Statistical Methods for Environmental Pollution Monitoring*. Van Nostrand Reinhold, New York, NY.

Holoubek, I., 2001. "Polychlorinated Biphenyl (PCB) Contaminated Sites Worldwide. In PCBs: Recent Advances in Environmental Toxicology and Health Effects," pp. 17–26, L.W. Robertson and L.G. Hansen, Eds., University of Kentukey Press, Lexington, KY.

Hopke, P.K., 1985. *Receptor Modeling in Environmental Chemistry*. Wiley-Interscience, New York, NY.

Hopke, P.K, K. Ito, T. Mar, W.F. Christensen, D.J. Eatough, R.C. Henry, E. Kim, F. Laden, R. Lall, T.V. Larson, H. Liu, L. Neas, J. Pinto, M. Stolzel, H. Suh, P. Paatero, and G.D. Thurston. "PM Source Apportionment and Health Effects: 1. Intercomparison of Source Apportionment Results," *J Expo Sci Environ Epidemiol.* **16**(3):275–86.

Imamoglu, I., K. Li, and E.R. Christensen, 2002. "Modeling Polychlorinated Biphenyl Congener Patterns and Dechlorination in Dated Sediment from the Ashtabula River, Ohio, USA." *Environ. Tox. Chem.* **21**(11):2283–2291.

Jarman, W.M., G.W. Johnson, C.E. Bacon, J.A. Davis, R.W. Risebrough, and R. Ramer. 1997. "Levels and Patterns of Polychlorinated Biphenyls in Water Collected from the San Francisco Bay and Estuary, 1993–1995," *Fresnius J. Anal. Chem.* **359**: 254–260.

Johnson, G.W., W.M. Jarman, C.E. Bacon, J.A. Davis, R. Ehrlich, and R.W. Risebrough, 2000. "Resolving Polychlorinated Biphenyl Source Fingerprints in Suspended Particulate Matter of San Francisco Bay," *Environ. Sci. Technol*. **34**:552–559.

Johnson, G.W., J.F. Quensen, J.R. Chiarenzelli, and M.C. Hamilton, 2006. "Polychlorinated Biphenyls." In *Environmental Forensics: Contaminant Specific Guide*. pp. 187–225. R. Morrison and B. Murphy, Eds., Elsevier. Amsterdam, The Netherlands.

Johnson, G.W., R. Ehrlich, W.Full, and S. Ramos. 2007. "Chapter 6: Principal components Analysis and Receptor Models in Environmental Forensics." In *An Introduction to Environmental Forensics.* 2nd Edition, pp. 207–272, R. Morrison and B. Murphy, Eds. Elsevier. Amsterdam, The Netherlands.

Magar, V.S., G.W. R.C. Johnson, J.F. Brenner. E.A. Quensen, G. Foote, J.A. Durell, J.A. Ickes, and C. Peven-McCarthy. 2005. "Long-term Recovery of PCB-contaminated Sediments at the Lake Hartwell Superfund Site: PCB Dechlorination. 1. End-member Characterization," *Environ Sci Technol*. **39**(10):3538–354747.

Leather, J.M., G. Durell, G.W. Johnson, and M. Mills, in prep. Integrated Forensics Approach to Fingerprint PCB Sources in Sediments. Final Report for ESTCP Project ER-0826. http://www.serdp.org/Program-Areas/Environmental-Restoration/

Contaminated-Sediments

Rachdawong, P. and E.R. Christensen, 1997. "Determination of PCB Sources by a Principal Component Method with Nonnegative Constraints," *Environ. Sci. Technol*. **31**:2686–2691.

Stout, S.A., J.M. Leather, and W.E. Corl, 2003. A User's Guide for Determining the Sources of Contaminants in Sediment: A Demonstration Study of PAH Sources in Sediments in the Vicinity of the Norfolk Naval Shipyard, Elizabeth River, Norfolk Virginia. SPAWAR Technical Report 1907. http://www.spawar.navy.mil/sti/publications/pubs/tr/1907/tr1907cond.pdf

APPENDIX A

List of Navy Activities with Metal, Bacteria, and PCB Impairments (NAVFAC, 2006)

CAUSE2004	State_Country2	Activity2004	WaterbodyName2004	Primary_Contaminant_Matrix200	IR_Related_Cont
Copper	IL	NAVSTA GREAT LAKES IL	Pettibone Creek	Sediment	No
Copper	FL	NAVSTA MAYPORT FL (FISC)	St. Johns above Trout River	Water	No
Copper	FL	NAS JACKSONVILLE FL	St Johns River above Warren Bridge	Water	No
Copper	FL	NAVSTA MAYPORT FL	St. Johns above Mouth	Water	No
Copper	FL	NAVSTA MAYPORT FL	St Johns above ICWW	Water	No
Copper	SC	NAVWPNSTA CHASN	Back River Reservoir in Forebay	Water	No
Copper	CA	NAS NORTH ISLAND SAN DIEGO CA (FRMER PHIBASE CORONADO)	San Diego Bay Shoreline: at Glorietta Bay	Water	No
Copper	CA	NAVWPNSTA SEAL BEACH	Huntington Harbour	Sediment	No
Copper	CA	NAVWPNSTA SEAL BEACH	Anaheim Bay	Sediment	No
Copper	CA	NAVWPNSTA SEAL BEACH (SAN PEDRO FUEL DEPOT)	Los Angeles Harbor - Main Channel	Tissue/Sed	No
Copper	CA	SUBASE SAN DIEGO CA	San Diego Bay Shoreline, America's Cup Harbor	Water	No
Copper	CA	NAVBASE VENTURA CTY PT MUGU CA	Calleguas Creek Reach 1 (was Mugu Lagoon on 1998 303d list	Tissue	Yes
Copper	CA	NAVSTA SAN DIEGO CA	San Diego Bay; between Sampson and 28th Streets	Sediment	No
Copper	CA	NAVSTA SAN DIEGO CA	Chollas Creek	Water	No
Copper	VA	NAVSUPPACT WASH (NSWC DAHLGREN)	Potomac River Middle Tidal	Water	No
Copper	MD	NAVSUPPACT WASH (NSWC INDIAN HEAD)	Potomac River Middle Tidal	Water	No
Copper	D.C.	NAVSUPPACT WASH (NAVSTA ANACOSTIA)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Sediment	No
Copper	D.C.	NAVSUPPACT WASH (NAVAL OBSERVATORY)	Lower Rock Creek DC	Water	No
Copper	D.C.	NAVSUPPACT WASH (WASHINGTON NAVY YARD)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Sediment	No
Zinc	IL	NAVSTA GREAT LAKES IL	Pettibone Creek	Sediment	No
Zinc	D.C.	NAVSUPPACT WASH (NAVSTA ANACOSTIA)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Sediment	No
Zinc	CA	NAVWPNSTA SEAL BEACH (SAN PEDRO FUEL DEPOT)	Los Angeles Harbor - Main Channel	Tissue/Sed	No
Zinc	CA	NAVBASE VENTURA CTY PT MUGU CA (PORT HUENEME)	Channel Islands Harbor	Sediment	No
Zinc	CA	NAVSTA SAN DIEGO CA	Chollas Creek	Water	No
Zinc	CA	NAVSTA SAN DIEGO CA	San Diego Bay; between Sampson and 28th Streets	Sediment	No
Zinc	CA	NAVBASE VENTURA CTY PT MUGU CA	Calleguas Creek Reach 1 (was Mugu Lagoon on 1998 303d list	Tissue	Yes
Zinc	D.C.	NAVSUPPACT WASH (NAVAL OBSERVATORY)	Lower Rock Creek DC	Water	No
Zinc	D.C.	NAVSUPPACT WASH (WASHINGTON NAVY YARD)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Sediment	No
Mercury	WV	NIOC SUGAR GROVE WV	South Fork South Branch Potomac River	Tissue	No
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	WA	NAVMEDCEN BREMERTON WA	Dyes Inlet	Tissue	Yes
Mercury	WA	NAVAL BASE KITSAP (JACKSON PARK HSG)	Dyes Inlet	Tissue	Yes
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	WA	NAVAL BASE KITSAP (SHIPYARD PUGET SOUND)	Sinclair Inlet	Sediment	Yes
Mercury	IL	NAVSTA GREAT LAKES IL	Pettibone Creek	Tissue	No
Mercury	SC	NAVWPNSTA CHASN	Cooper River at Bushy Park	Tissue	No
Mercury	GA	SUBASE KINGS BAY GA	St. Marys River (Georgia List)	Tissue	No
Mercury	FL	NAS PENSACOLA FL	Bayou Grande	Tissue	No
Mercury	FL	NAS PENSACOLA FL	Pensacola Bay	Tissue	No
Mercury	FL	NAS PENSACOLA FL	Navy Point (Pensacola Bay)	Tissue	No
Mercury	FL	NAS PENSACOLA FL (CORRY STATION)	Bayou Chico	Tissue	No
Mercury	FL	NAS PENSACOLA FL (CORRY STATION)	Bayou Chico Beach	Sediment	No
Mercury	FL	NAS WHITING FLD MILTON FL	Blackwater River	Tissue	No
Mercury	SC	NAVWPNSTA CHASN	Back River Reservoir	Tissue	No
Mercury	SC	NAVAL WEAPONS STATION CHASN (SHORT STAY)	Lake Moultrie at Dam	Tissue	No
Mercury	CA	NAVWPNSTA SEAL BEACH (DET CONCORD)	Suisun Bay	Tissue/Sed	No
Mercury	CA	CSO FISC OAKLAND CA (RICHMOND PT MOLATE)	San Francisco Bay	Tissue/Sed	No
Mercury	CA	NAVBASE VENTURA CTY PT MUGU CA	Calleguas Creek Reach 1 (was Mugu Lagoon on 1998 303d list	Tissue	No
Mercury	CA	CSO NS TREASURE ISLAND CA	San Francisco Bay	Tissue/Sed	No
Mercury	CA	NAVWPNSTA SEAL BEACH (FALLBROOK CALIFORNIA)	Santa Margarita River	Tissue	No
Mercury	CA	CSO FISC OAKLAND CA (ALAMEDA ANNEX)	San Francisco Bay	Tissue/Sed	No
Mercury	CA	NAF EL CENTRO CA	New River	Tissue/Sed	No
Mercury	NV	NAS FALLON NV	Stillwater Marsh	Tissue	No
Mercury	CA	CSO FISC OAKLAND CA (ALAMEDA FACILITY)	San Francisco Bay	Tissue/Sed	No
Mercury	CA	CSO FISC OAKLAND CA	San Francisco Bay	Tissue/Sed	No
Mercury	CA	CSO HUNTERS POINT ANNEX	San Francisco Bay	Tissue/Sed	No
Mercury	CA	NAS LEMOORE CA (STOCKTON)	Delta Waterways	Tissue/Sed	No
Mercury	CA	NAVSTA SAN DIEGO CA	San Diego Bay; between Sampson and 28th Streets	Sediment	No
Mercury	CA	CSO NAS ALAMEDA CA	San Francisco Bay	Tissue/Sed	No
Mercury (met	WV	ALLEGANY BALLISTICS LAB	North Branch Potomac River	Tissue	No

Navy Bases with Metal Impairments

CAUSE2004	State_Country2004	Activity2004	WaterbodyName2004	Primary_Contaminant_Matrix2004	IR_Related_Contaminant2004
Bacteria	Guam	NAVBASE GUAM (BARRIGADA)	Northern Guam Lens Aquifer	Water	No
Bacteria	Guam	NAVBASE GUAM (FINEGAYAN)	Northern Guam Lens Aquifer	Water	No
Bacteria	TX	NAS CORPUS CHRISTI TX	Oso Creek	Water	No
Bacteria	TX	NAS CORPUS CHRISTI TX (ALF CABANISS)	Oso Creek	Water	No
Bacteria	TX	NAS JRB FT WORTH TX	West Fork Trinity River	Water	No
Bacteria	FL	NAS PENSACOLA FL	Navy Point (Pensacola Bay)	Water	No
Bacteria	FL	NAS PENSACOLA FL (CORRY STATION)	Bayou Chico Beach	Water	No
Bacteria	FL	NAVSUPPACT PANAMA CITY	St. Andrews Bay	Water	No
Bacteria	TX	NAS CORPUS CHRISTI TX	Oso Bay	Water	No
Bacteria	CA	NAVSTA SAN DIEGO CA	Chollas Creek	Water	No
Bacteria	CA	NAVBASE VENTURA CTY PT MUGU CA (PORT HUENEME)	Channel Islands Harbor	Water	No
Bacteria	CA	NAF EL CENTRO CA	New River	Water	No
Bacteria	D.C.	NAVSUPPACT WASH (NAVAL RESEARCH LAB)	Lower Potomac DC (Hains Point to Woodrow Wilson Bridg	Water	No
Bacterial Indicators	CA	NAS NORTH ISLAND SAN DIEGO CA (IMPERIAL BEACH)	Tijuana River Estuary	Water	No
Coliforms	FL	NAS PENSACOLA FL (SAUFLEY FIELD)	Elevenmile Creek	Water	No
Coliforms	FL	NAS PENSACOLA FL (SAUFLEY FIELD)	Eightmile Creek	Water	No
E. Coli	IN	NAVSUPPACT CRANE	First Creek	Water	No
E. Coli	IL	NAVSTA GREAT LAKES IL	Lake Michigan Beaches	Water	No
E. Coli	IN	NAVSUPPACT CRANE	Boggs Creek	Water	No
E. Coli	IN	NAVSUPPACT CRANE	Rocky Branch and other tributaries	Water	No
E. Coli	TN	NAVSUPPACT MIDSOUTH MEMPHIS TN	Big Creek	Water	No
E. Coli	TN	NAVSUPPACT MIDSOUTH MEMPHIS TN (NSWC CARDEROCK DIV)	McKellar Lake	Water	No
Fecal Coliform	VA	NAVWPNSTA YORKTOWN (CHEATHAM ANNEX)	King Creek	Water	No
Fecal Coliform	WV	ALLEGANY BALLISTICS LAB	North Branch Potomac River	Water	No
Fecal Coliform	VA	NAS OCEANA VA	West Neck Creek (Upper) to London Bridge Creek	Water	No
Fecal Coliform	VA	NAS OCEANA VA	London Bridge Creek & Canal #2	Water	No
Fecal Coliform	VA	NAVWPNSTA YORKTOWN	King Creek	Water	No
Fecal Coliform	VA	NAVSUPPACT NORFOLK NAVSHIPYD (□SOUTH GATE)	Southern Branch, Elizabeth River	Water	No
Fecal Coliform	WV	NIOC SUGAR GROVE WV	South Fork South Branch Potomac River	Water	No
Fecal Coliform	VA	NAVSUPPACT NORFOLK NAVSHIPYD	Southern Branch, Elizabeth River	Water	No
Fecal Coliform	VA	NAVPHIBASE LITTLE CREEK VA (WALLOPS ISLAND VA)	Little Mosquito Creek	Water	No
Fecal Coliform	ME	NAVSUPPACT PORTSMOUTH NAVSHIPYD	Piscataqua River Estuary	Water	No
Fecal Coliform	NJ	NAVWPNSTA EARLE NJ (WATERFRONT EARLE)	Town Brook	Water	No
Fecal Coliform	WA	NAVAL BASE KITSAP (KEYPORT NUWC)	Liberty Bay	Water	No
Fecal Coliform	WA	NAVAL BASE KITSAP (SUBASE Bangor)	Clear Creek (west fork)	Water	No
Fecal Coliform	WA	NAVAL BASE KITSAP (MANCHESTER WA)	Beaver Creek	Water	No
Fecal Coliform	SC	NAVWPNSTA CHASN	Foster Creek at Charleston CPW Intake	Water	No
Fecal Coliform	SC	NAVWPNSTA CHASN	Goose Creek at Station MD-039	Water	No
Fecal Coliform	GA	NAS ATLANTA GA	Nickajack Creek	Water	No
Fecal Coliform	GA	NAS ATLANTA GA	Chattahoochee River	Water	No
Fecal Coliform	FL	NAVSTA MAYPORT FL (FISC)	Trout River	Water	No
Fecal Coliform	FL	NAS PENSACOLA FL	Navy Point (Pensacola Bay)	Water	No
Fecal Coliform	FL	NAS PENSACOLA FL (CORRY STATION)	Jones Creek	Water	No
Fecal Coliform	FL	NAS PENSACOLA FL (CORRY STATION)	Bayou Chico	Water	No
Fecal Coliform	FL	NAS PENSACOLA FL (CORRY STATION)	Bayou Chico Beach	Water	No
Fecal Coliform	FL	NAVSTA MAYPORT FL	Sherman Creek	Water	No
Fecal Coliform	FL	NAVSUPPACT PANAMA CITY	St. Andrews Bay	Water	No
Fecal Coliform	FL	NAS WHITING FLD MILTON FL	Big Coldwater Creek	Water	No
Fecal Coliform	FL	NAS JACKSONVILLE FL (OLF WHITEHOUSE)	McGirts Creek	Water	No
Fecal Coliform	MD	NATNAVMEDCEN BETHESDA MD	Rock Creek	Water	No
Fecal Coliform	MD	NAS PATUXENT RIVER MD	Patuxent River Lower	Water	No
Fecal Coliform	MD	NAS PATUXENT RIVER MD (WEBSTER FIELD)	St. Marys River	Water	No
Fecal Coliform	MD	NAS PATUXENT RIVER MD (SOLOMONS ISLAND MD)	Patuxent River Lower	Water	No
Fecal Coliform	D.C.	NAVSUPPACT WASH (NAVSTA ANACOSTIA)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Water	No
Fecal Coliform	D.C.	NAVSUPPACT WASH (NAVAL OBSERVATORY)	Lower Rock Creek DC	Water	No
Fecal Coliform	D.C.	NAVSUPPACT WASH (NAVAL OBSERVATORY)	Dunbarton Oaks	Water	No
Fecal Coliform	D.C.	NAVSUPPACT WASH (WASHINGTON NAVY YARD)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Water	No
Fecal Coliforms	FL	NAS JACKSONVILLE FL	Ortega River	Water	No

Navy Bases with Microbial Impairment

CAUSE2004	State_Country2004	Activity2004	WaterbodyName2004	Primary_Contaminant_Matrix2004	IR_Related_Contaminant2004
PCBS	VA	NAVWPNSTA YORKTOWN (CHEATHAM ANNEX)	King Creek (Mouth)	Tissue	No
PCBS	VA	NAVWPNSTA YORKTOWN (□YORKTOWN FUEL DEPOT)	Wormley Creek	Tissue	No
PCBS	ME	NAS BRUNSWICK ME	Androscoggin River	Tissue	No
PCBS	PA	NAVSUPPACT MECHANICSBURG PA	Trindle Spring Run	Tissue	No
PCBS	VA	NAVWPNSTA YORKTOWN	King Creek (Mouth)	Tissue	No
PCBS	VA	NAVPHIBASE LITTLE CREEK VA	Little Creek Channel	Tissue	No
PCBS	PA	NAS JRB WILLOW GROVE PA	Park Creek	Tissue	No
PCBS	PA	NAVSUPPACT MECHANICSBURG PA (DET PHIL ANNEX PNBC)	Schuylkill River	Tissue	Yes
PCBS	VA	NAVSUPPACT NORFOLK NAVSHIPYD (□ST JULIENS CREEK ANNEX)	Saint Julian Creek	Tissue	No
PCBS	PA	NAVSUPPACT MECHANICSBURG PA (DET PHIL ANNEX PNBC)	Delaware River	Tissue	Yes
PCBS	PA	NAS JRB WILLOW GROVE PA	Little Neshaminy Creek	Tissue	No
PCBS	D.C.	NAVSUPPACT WASH (NAVAL OBSERVATORY)	Dunbarton Oaks	Tissue	No
PCBS	HI	NAVSTA PEARL HARBOR HI	Pearl Harbor	Tissue	Yes
PCBS	HI	NAVSTA PEARL HARBOR (NAVSHIPYD)	Pearl Harbor	Tissue	Yes
PCBS	HI	NAVSTA PEARL HARBOR HI (□PEARL CITY)	Pearl Harbor	Tissue	Yes
PCBS	HI	NAVSTA PEARL HARBOR HI (□EWA)	Pearl Harbor	Tissue	Yes
PCBS	HI	NAVSTA PEARL HARBOR HI□ (WAIPAHU)	Pearl Harbor	Tissue	Yes
PCBS	IL	NAVSTA GREAT LAKES IL	Pettibone Creek	Sediment	No
PCBS	IL	NAVSTA GREAT LAKES IL	Lake Michigan	Tissue	No
PCBS	TX	NAS JRB FT WORTH TX	West Fork Trinity River	Tissue	No
PCBS	TX	NAS JRB FT WORTH TX	Lake Worth	Tissue	No
PCBS	IL	NAVSTA GREAT LAKES IL	South Branch Pettibone Creek	Sediment	No
PCBS	IL	NAVSTA GREAT LAKES IL	Lake Michigan Beaches	Tissue	No
PCBS	TN	NAVSUPPACT MIDSOUTH MEMPHIS TN (NSWC CARDEROCK DIV)	Mississippi River @ Memphis, TN	Sediment	No
PCBS	TN	NAVSUPPACT MIDSOUTH MEMPHIS TN (NSWC CARDEROCK DIV)	McKellar Lake	Sediment	No
PCBS	CA	CSO NS TREASURE ISLAND CA	San Francisco Bay	Tissue/Sed	No
PCBS	CA	NAVSTA SAN DIEGO CA	San Diego Bay	Tissue	No
PCBS	CA	CSO FISC OAKLAND CA (RICHMOND PT MOLATE)	San Francisco Bay	Tissue/Sed	No
PCBS	CA	NAVBASE VENTURA CTY PT MUGU CA	Calleguas Creek Reach 1 (was Mugu Lagoon on 1998 303d list	Tissue	Yes
PCBS	CA	NAVWPNSTA SEAL BEACH	Anaheim Bay	Tissue	No
PCBS	CA	CSO FISC OAKLAND CA	San Francisco Bay	Tissue/Sed	No
PCBS	CA	CSO FISC OAKLAND CA (ALAMEDA ANNEX)	San Francisco Bay	Tissue/Sed	Yes
PCBs	CA	SUBASE SAN DIEGO CA	San Diego Bay	Tissue	No
PCBs	CA	NAVMEDCEN SAN DIEGO CA	San Diego Bay	Tissue	No
PCBs	CA	NAF EL CENTRO CA	New River	Tissue/Sed	No
PCBS	CA	NAVWPNSTA SEAL BEACH	Huntington Harbour	Tissue	No
PCBS	CA	NAVBASE VENTURA CTY PT MUGU CA	Calleguas Creek Reach 2 (Estuary to Protrero Rd)	Tissue	No
PCBS	CA	CSO HUNTERS POINT ANNEX	San Francisco Bay	Tissue/Sed	No
PCBS	CA	CSO NAS ALAMEDA CA	San Francisco Bay	Tissue/Sed	No
PCBS	CA	NAVWPNSTA SEAL BEACH (DET CONCORD)	Suisun Bay	Tissue/Sed	No
PCBS	CA	CSO FISC OAKLAND CA (ALAMEDA FACILITY)	San Francisco Bay	Tissue/Sed	No
PCBs	CA	NAS NORTH ISLAND SAN DIEGO CA (FRMER PHIBASE CORONADO)	San Diego Bay	Tissue	No
PCBs	CA	NAS NORTH ISLAND SAN DIEGO CA (IMPERIAL BEACH)	Pacific Ocean Shoreline - Imperial Beach Pier	Tissue	No
PCBS	CA	NAVBASE VENTURA CTY PT MUGU CA (PORT HUENEME)	Port Hueneme Harbor	Tissue	No
PCBs	CA	NAVWPNSTA SEAL BEACH (SAN PEDRO FUEL DEPOT)	Los Angeles Harbor - Main Channel	Tissue/Sed	No
PCBS	CA	NAS NORTH ISLAND SAN DIEGO CA	San Diego Bay	Tissue	No
PCBS	MD	NAS PATUXENT RIVER MD (WEBSTER FIELD)	St. Marys River	Tissue	No
PCBs	D.C.	NAVSUPPACT WASH (WASHINGTON NAVY YARD)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Tissue	Yes
PCBs	MD	NAVSUPPACT WASH (USNA ANNAPOLIS)	Severn River	Tissue	No
PCBS	MD	NAVSUPPACT WASH (NAVSTA)	Severn River	Tissue	No
PCBS	VA	NAVSUPPACT WASH (NSWC DAHLGREN)	Potomac River Middle Tidal	Tissue	No
PCBS	MD	NAVSUPPACT WASH (NSWC INDIAN HEAD)	Potomac River Middle Tidal	Tissue	No
PCBs	D.C.	NAVSUPPACT WASH (NAVSTA ANACOSTIA)	Lower Anacostia DC (Below Pennsylvania Ave Bridge)	Tissue	No
PCBS (Dioxin-Like	CA	CSO FISC OAKLAND CA (ALAMEDA ANNEX)	San Francisco Bay	Tissue/Sed	No
PCBS (Dioxin-Like	CA	CSO FISC OAKLAND CA	San Francisco Bay	Tissue/Sed	Yes
PCBS (Dioxin-Like	CA	CSO FISC OAKLAND CA (ALAMEDA FACILITY)	San Francisco Bay	Tissue/Sed	Yes
PCBS (Dioxin-Like	CA	NAVWPNSTA SEAL BEACH (DET CONCORD)	Suisun Bay	Tissue/Sed	No
PCBS (Dioxin-Like	CA	CSO NAS ALAMEDA CA	San Francisco Bay	Tissue/Sed	Yes
PCBS (Dioxin-Like	CA	CSO NS TREASURE ISLAND CA	San Francisco Bay	Tissue/Sed	Yes
PCBS (Dioxin-Like	CA	CSO HUNTERS POINT ANNEX	San Francisco Bay	Tissue/Sed	Yes
PCBS (Dioxin-Like	CA	CSO FISC OAKLAND CA (RICHMOND PT MOLATE)	San Francisco Bay	Tissue/Sed	Yes

Navy Bases with PCB Impairment

APPENDIX B

Case Study on Source Tracking for Bacteria

Case Study on Naval Air Station Oceana/ Chowan River Bacterial Source Tracking (BST) TMDL Study - Completed Jan, 2007.

These comments are general overall comments applicable to this particular case study; but these comments are meant to serve as lessons learned in a BST study and can be applied to other bacterial TMDL investigations to improve upon future endeavors. For more specific information on this study (~100 page report), please e-mail either the author of this review, Y.M. Arias-Thode at meriah.ariasthode@navy.mil or Dave Cotnoir at david.cotnoir@navy.mil.

Following is a synopsis of this particular study taken directly from the executive summary in the report entitled, Investigation of Bacterial Sources in the Outfall 001 and 006 Watersheds. Naval Air Station Oceana, Virginia Beach, Virginia. "The Virginia Department of Environmental Quality (VADEQ) listed (Naval Air Station Oceana) as being unable to attain the primary contact recreation use due to violations of the bacterial water quality standard and has therefore developed a Total Maximum Daily Load (TMDL). Based on water quality modeling and limited sampling, the TMDL report identified Naval Air Station (NAS) Oceana, specifically the …Horse Stables, as a significant contributor to the bacterial impairment – 92% of the load. To characterize bacterial contributions from the stables, the Navy hired a corporation to conduct an investigation of bacterial sources in two outfalls. In order to quantify the relative contribution of bacteria through bacterial enumerations and bacterial source tracking, monthly sampling was performed over 10-month period and intensive sampling was performed three times a week in historical wet months." [This is a summary of four paragraphs in the executive summary and some words were changed, but the gist is generally the same].

General comments regarding the aforementioned report are:
(1.) From this particular study, it was observed that horse manure is applied to 3 acres of land in one of the watersheds (Outfall 006). Questions that were not addressed are: Is this a necessary fertilizer? Can less be used and the same task still accomplished as part of a best management practice (BMP)? More importantly, is it possible to compost the horse manure with sufficient heat to kill off any bacteria prior to using as a fertilizer?
(2.) Two different contractors performed an Antibiotic Resistance Analysis (ARA) and the results were very different. One contractor implicated the Navy, and the other did not. However, the differences were not adequately addressed. It may be that the initial contractor did not take sufficient samples, or that their methodology was deficient or not effective. Due to the differences in study outcomes, more of a discussion would be effective as a lessons learned and means to improve future studies.
(3.) Some specific suggestions were provided to the Navy by the contractors regarding the implementation of some best practice management options that were recommended in a report by the City of Virginia such as "Rooftop Runoff Collection for Horse Stables, an Animal Waste Management Plan for Oceana, and an Equine Facility Inventory for Virginia Beach." The NAS Oceana BST study accepted these recommendations, but there was no mention as to what the procedures were specifically or whether the procedures were implemented before, during, or after the study. It is important to know if

these suggestions worked well or if differences were observed prior to or after the implementation. This information would serve others well that may have similar issues. These appear to be great recommendations for BMP's.

(4.) In this particular BST project, 24 isolates were chosen as the sample size and examined via the ARA technique.. Unfortunately, dependent on the dilution factor, 24 isolates were not obtained and the results were not statically valid. A future recommendation would be to do more than 24 isolates; even 50, or 100 for statistical purposes. The ARA technique is not very accurate and to include data with insufficient numbers of isolates, the data does not provide a high confidence level. More samples would provide a higher confidence in the data. A recommendation to increase the number of isolates and not have the issue of 'insufficient isolates obtained' is to have the investigators plate 3 dilutions; the dilution of interest and also the dilution above and below the presumed dilution (bacterial counts are always approximate). This will aid in obtaining the requisite number of bacterial colonies (24, 50, or 100) necessary for the BST analysis and necessary for statistical substantiation.

A secondary recommendation regarding working with small numbers of isolates is even if you have low numbers of bacterial colonies, BST can still be performed. The data may be important in that, for example, the analysis of the bacteria may implicate a specific site (for example, a nearby poultry farm). However, on occasion, when the bacterial numbers are high, it is then that inputs are observed from a secondary source (for example, horse stables). This information will tell you that there is an intermittent problem and it may be easier to determine the reason for the problem. In this particular example regarding a horse stable, the secondary source contamination could be due to rain, or perhaps the horses were washed that day or the stables cleaned. This type of information would help in the establishment of future BMP's.

(5.) In this particular study, comments were made that the study was not performed during the high precipitation months. It would be good to explain the reasons for this decision so that others attempting to perform a BST can learn from this experience. Also, these comments might include information regarding whether BST is or is not recommended during high precipitation events.

(6.) Lastly, in this particular study, only two potential sources were analyzed (livestock vs. other). The drawback to only looking at two potential sources is that you gain no more information other than is it, or is not, livestock. This type of information makes it difficult to suggest future best management practices. One would assume if there is a TMDL exceedance from a specific site, that the party is still responsible. More information would help establish true responsibility, as well as future endeavors to prevent TMDL's from occurring.

APPENDIX C

Case Study on Pollutant Source Tracking for Metals (Cu)

Case Study Report on Fingerprinting Sources of Metals in Coastal Environments

Three different approaches are presented for discrimination of metal sources:

(i) statistical analysis of metal concentration gradients in surface water,

(ii) isotopic measurements in sediments, and

(iii) ratios of stable isotopes in waters and sediments.

The information used for these case studies is from previous and recent efforts for environmental assessment in San Diego Bay. The goal of presenting these results in this report is to demonstrate the methodology for using these approaches to identify potential metal sources.

Statistical Analysis of Metal Concentration Gradients in Surface Waters of San Diego Bay. This approach builds upon the extensive sampling performed under Strategic Environmental Research and Development Program (SERDP) Project CP-1156, on "*Determining the Fate and Ecological Effects of Copper and Zinc Loading in Estuarine Environments: A Multi-Disciplinary Program*" (Chadwick *et al*., 2005; http://www.serdp.org/Research/upload/CP_1156_FR.pdf). This effort was conducted in San Diego Bay from August 2000 to December 2004, with the main goals of establishing copper budgets, understanding copper and zinc speciation, and evaluating toxicity. This approach required simultaneous collection of circulation, hydrographic, water quality, copper (Cu), zinc (Zn), and biological data, at the appropriate spatial and temporal scales necessary to understand the processes controlling metal distributions and toxicities in the bay. These data are interpreted in the context of locating metal sources within the bay, although as described below the compositing of samples within large portions of the bay may marginally reduce their value to some extent for fingerprinting exact source locations.

San Diego Bay is considered a prototype harbor system, as it provides a unique range of hydrological conditions with a relatively persistent spatial distribution of copper and zinc concentrations, and well-defined chronic sources of copper. For purposes of the SERDP study, the bay was divided into 25 boxes of about 1 km scale, expanding throughout the main body of the bay, and with a dedicated box to describe each of two semi-enclosed marinas within the bay, Shelter Island (box 6) and Commercial Basin (box 9; Figure 1). Because of the objectives of project CP-1156, integrated samples were obtained at each of these boxes by continuous collection of surface water (about 1 m deep) into a single large container, while traveling in a well-defined path throughout each box (Figure C-1). Sampling from the fully-collected integrated sample commenced as soon as the transect for each box was completed. Thus, the spatial resolution is dictated by the size of the boxes, and provides metal concentration gradients that are representative of processes and sources affecting larger areas within the bay (i.e., encompassing a whole box or several boxes), in comparison to delineation of single-point sources within each of the boxes. Despite this decreased resolution, the data and samples collected for CP-1156 provide the information required for the differentiation of zones within San Diego Bay affected by specific sources of the suite of metals reported here.

Samples from the event of 30 August 2000 were analyzed for a suite of 15 elements to ascertain both total and dissolved (i.e., filtered through 0.45µm pore size) concentrations.

Analysis was performed using ICP-MS following a solvent-solvent preconcentration (Bruland *et al*., 1985). Spatial concentration gradients of dissolved metals grouped according to their concentration range are shown in Figure C-2. Metals in the concentration range of < 0.02 µg/L are Arsenic (As), Chromium (Cr), Beryllium (Be), Silver (Ag), and Thallium (Tl), whereas those metals in the concentration range of 0.02 to 0.3 µg/L include Barium (Ba), Cadmium (Cd), Cobalt (Co) and Lead (Pb). Metals in the concentration range 0.3 to 8 µg/L (parts per billion; ppb) are Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Vanadium (V) and Zinc (Zn).

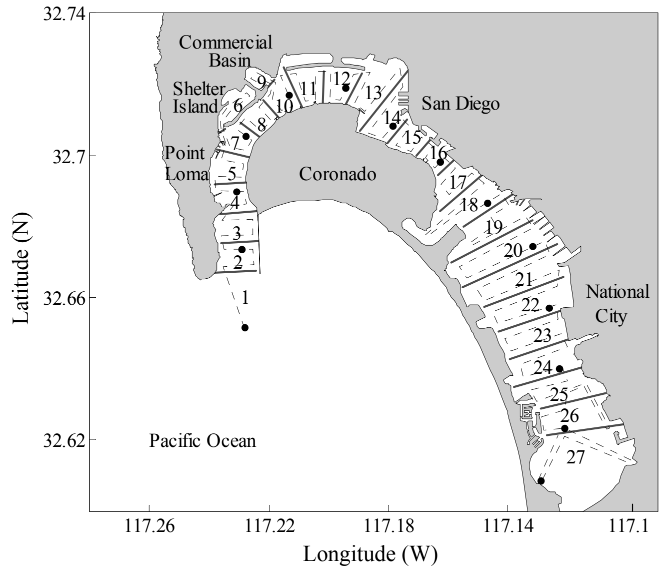


Figure C-1. Boxes and transect path for the determination of fate and effects of copper and zinc (SERDP CP-1156) in San Diego Bay (from: Chadwick *et al*., 2005).

Spatial distributions are affected by source inputs, circulation and residence time within the bay. In general, most metal distributions tend to increase from the mouth (box 1) to the head (box 27) of the bay. Salinity has a similar trend (not shown), resulting from the increase in residence time at the head of the bay. Metals with conservative distribution (i.e., similar concentrations throughout the bay) include As, Ba, Tl and V. Both Cu and Zn show greater concentrations in Shelter Island (box 6) and Commercial Basin (box 9), marinas known to have significant anthropogenic inputs of these metals and restricted circulation.

Another representation of the data is provided in Figure C-3, as a contour distribution of total copper (µg/L) predicted with the fate and transport model, Curvilinear Hydrodynamics in Three Dimensions (CH3D). This predicted distribution provides a snapshot of the areas affected by copper in the bay. The most striking features in Figure C-3 are the high concentration levels of copper in areas affected by source inputs with restricted circulation (i.e., marinas and harbors), and in areas with source inputs, good circulation, and relatively high residence time, as in the central portion of the bay, where some of the Navy installations are disbursed with mostly private industrial operations. This predicted distribution also indicates the effect of tidal pumping of coastal waters, which recirculates these low-metal concentration waters at the mouth of the bay, with an effect that extends to boxes 7 and 8 in the Bay adjacent to Shelter Island. In contrast,

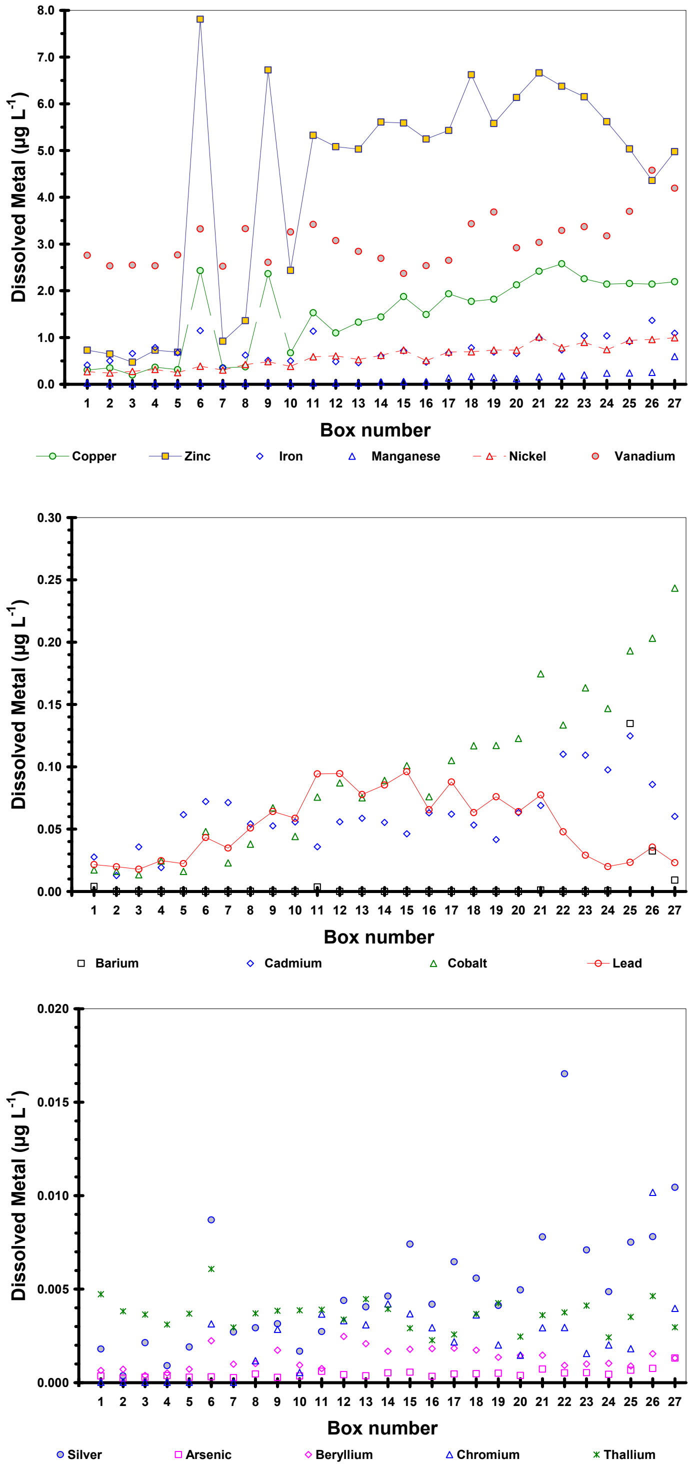


Figure C-2. Concentration gradients for 15 different metals in San Diego Bay for 30 August 2000. For clarity, the metals are grouped depending on their concentration range as noted on the y-axis. Box numbers correspond to those in Figure C-1, and follow a spatial distribution between the mouth and the head of the bay (box 1 and 27, respectively) at about 1 Km intervals.

total copper concentrations in the head of the Bay (box 27) exhibit the same level as those found in coastal waters, in spite of the increased residence time estimated to these areas of the bay.

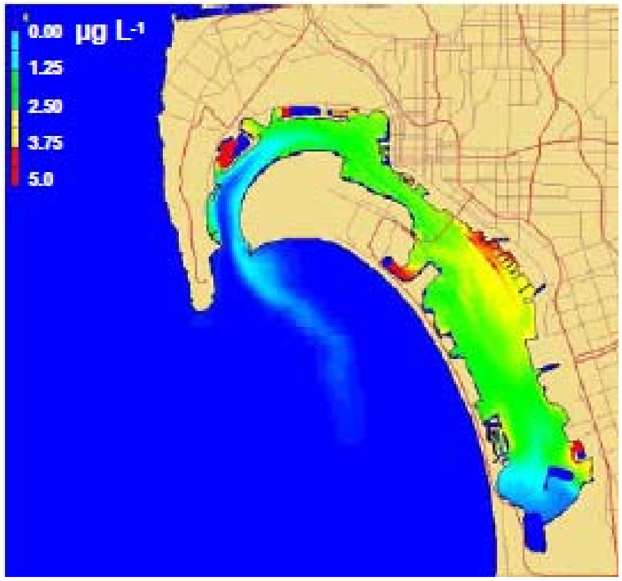


Figure C-3. Contour distribution for total copper (µg/L) in San Diego Bay predicted using the data from 30 August 2000. The distribution was predicted with the fate and transport model CH3D (from Chadwick *et al*., 2005). Note the concentration scale from low values (blue) to high values (red).

In addition to viewing concentration gradients in contour maps, one can also look for common correlation patterns amongst various metals with statistical multivariate analysis, an approach frequently used to discern common contaminant sources. The metal concentrations measured by ICP-MS and 29 environmental parameters reported by Project CP-1156 (Chadwick et al., 2005; Table 1) were analyzed by Principal Component Analysis (PCA). Due to the limited resolution of the integrated samples, instead of defining sources localized within any box, the statistical analysis provided information on areas with different levels of metals, resulting from the complex interaction between sources and natural physical, chemical and biological processes in the bay. Furthermore, for clarity considerations, the statistical analysis focused on dissolved Cu, Zn, Ni and Pb (the four metals with regulatory relevancy), and a parameter that represents the concentration distribution within the bay, salinity.

PCA is a statistical tool with applications in diverse fields of science, from social sciences, marketing, and computer graphics, to neuroscience and environmental research, for extracting relevant information from complex datasets. PCA is a simple, non-parametric method that is applicable to most datasets, independent of how the data were acquired. The goal of PCA is to describe the information using a minimal number of parameters, termed principal components, and to enable identification of simple underlying structures (dependencies) in the dataset. This allows one to reduce the dimensionality of the dataset, or to explain most of the variability of the data with a reduced number of principal components compared with the number of original variables. This is achieved by evaluating the degree to which some of the parameters can be

estimated from other parameters in the dataset. The basic assumption is that there is a linear correlation between some of the parameters, which is explored with correlation factors, the first step in PCA analysis. This information is then used to quantify principal components via linear algebra, which are described using their corresponding eigenvalues and eigenvectors, where large eigenvalues (> 1.00) typically represent parameters with more variability in the data. Principal components are considered non-detectable, uncorrelated latent variables, meaning these are not real environmental parameters, but they can define the cumulative effect of several real environmental parameters (sources). The common correlation pattern in each principal component is interpreted to represent a particular source; however, it can be sometime difficult to decide what source is represented by a particular principal component, and how many principal components (representing different sources) are present within the dataset. Principal components with eigenvalues greater than 1.00 are considered significant for reasons mentioned above, while those with eigenvalues lower than 1.00 are considered less significant, as they do not explain a significant portion of the dataset variability. These eigenvalues are used to provide a simple description of the data by plotting the principal components in loading and scores plots. For example, in San Diego Bay, the loading plot provides an indication of the parameter that drives the data to a specific region within the scores plot. Moreover, the scores plot provides the effect (impact) of the loading sources on the different boxes sampled in San Diego Bay.

The correlation factors calculated for San Diego Bay are shown in Table C-2. The high correlation coefficient between Cu and Zn indicates that they may have the same source or their sources may be co-located. Notice that both Ni and Pb have lower correlation coefficients, this is due to differences in their concentration gradients in the bay. For the concentration gradients of the metals analyzed by PCA (highlighted with lines connecting the data in Figure C-2), the similarity in the distributions of Cu and Zn is noticeable. As indicated above, and confirmed by correlation analysis, the spatial distribution of salinity is similar to the gradients of Cu and Zn. In comparison, Ni has a less pronounced increase in concentration going from the mouth (box 1) to the back (box 27) of the bay, with less correlation to salinity. Finally, Pb shows a completely different gradient, with higher concentrations in the middle of the bay, and a negative correlation to salinity. In spite of the data complexity, PCA successfully defines a minimal number of eigenvalues that represent the information (Table C-2), with two eigenvalues greater than 1.00, representing a significant portion (82%) of the variability in the dataset. For this reason, the following discussion of loading and scores plots is restricted to only the first two principal components.

The loading plot (Figure C-4) for this information illustrates the effect of Pb, Zn and Cu sources, and salinity (i.e., proxy for residence time), on driving the data to specific areas of the plot of principal component 2 (y-axis) versus principal component 1 (x-axis). The score plot (Figure C-5) shows how different portions of the bay are impacted by either sources of metals, or residence time. Areas affected by coastal water (boxes 1 to 8; quadrant 3, Q3) are not significantly impacted by metal sources within the bay or by the residence time. Boxes 10 to 12 (Q1) are impacted more by sources of Pb. Boxes 13 to 20, and the marinas in particular, boxes 6 and 9, show more impact due to sources of Cu and Zn (Q2). The remaining boxes (22 to 27) are mostly impacted by salinity (i.e., longer residence time in the head of the bay; Q4). PCA analysis indicates that sources of Ni do

not have a significant impact, in comparison with the sources of other metals evaluated. The loading plot shows that Ni should drive the data to the lower central area of the plot (between Q3 and Q4); however, the score plot does not show any information in that area, indicating a minimal impact due to Ni sources.

The analysis of metal gradients presented here shows the approach for identification of sources. As indicated above, the data used for this example is not perfectly suited for fingerprinting specific sources. However, using the appropriate sampling design for each specific site should provide positive identification and of sources in that site.

Table C-1. Multivariate correlations or correlation coefficients among the different parameters analyzed with PCA.

	Salinity	**Dissolved Cu**	**Dissolved Zn**	**Dissolved Ni**	**Dissolved Pb**
Salinity	1.000				
Dissolved Cu	0.679	1.000			
Dissolved Zn	0.353	0.828	1.000		
Dissolved Ni	0.369	-0.195	-0.423	1.000	
Dissolved Pb	-0.653	-0.330	0.092	-0.074	1.000

Table C-2. Eigenvalues and percent of prediction of the variance in the data for dissolved Cu, Ni, Pb, Zn and salinity measured in San Diego Bay on 30 August 2000.

Number	**Eigenvalue**	**Percent**	**Percent**	**Cumulative percent**
1	2.454	49.08		49.08
2	1.645	32.91		81.99
3	0.779	15.58		97.57
4	0.082	1.63		99.20
5	0.040	0.80		100.00

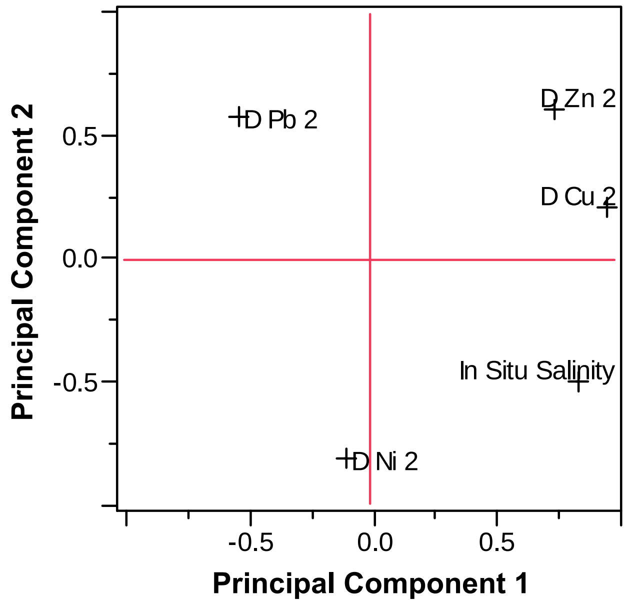


Figure C-4. Loading plot for Principal Component Analysis of dissolved Cu, Ni, Pb, Zn and salinity measured on 30 August 200 in San Diego Bay.

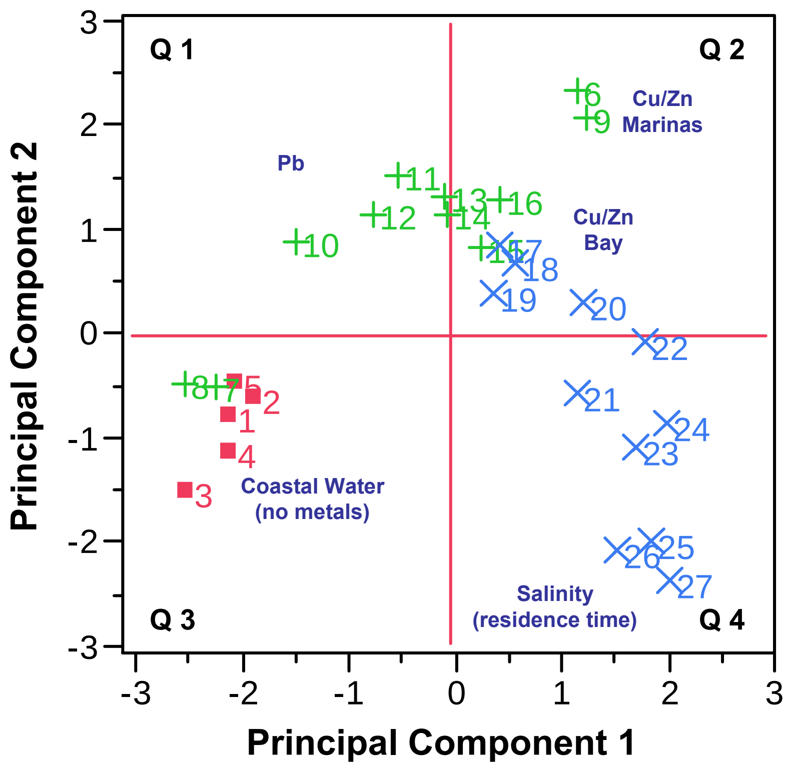


Figure C-5. Score plot for Principal Component Analysis of dissolved Cu, Ni, Pb, Zn and salinity measured on 30 August 200 in San Diego Bay. The quadrants are identified by Q#, numbers correspond to boxes used to describe the Bay, and different symbols and colors were used for the groups of boxes for clarity in the discussion.

Isotopic Measurements in Sediments of San Diego Bay. This approach is based on sediment data collected in support of a Total Maximum Daily Load (TMDL) study in Paleta Creek (Katz 2005; Figure 6). The sediments were collected from the bay area by Mole Pier, and from areas near Installation Restoration (IR) areas 2, 3 and 4 from upstream locations within Paleta Creek. The objective of the study was to provide information on chemistry levels upstream from the TMDL study area.

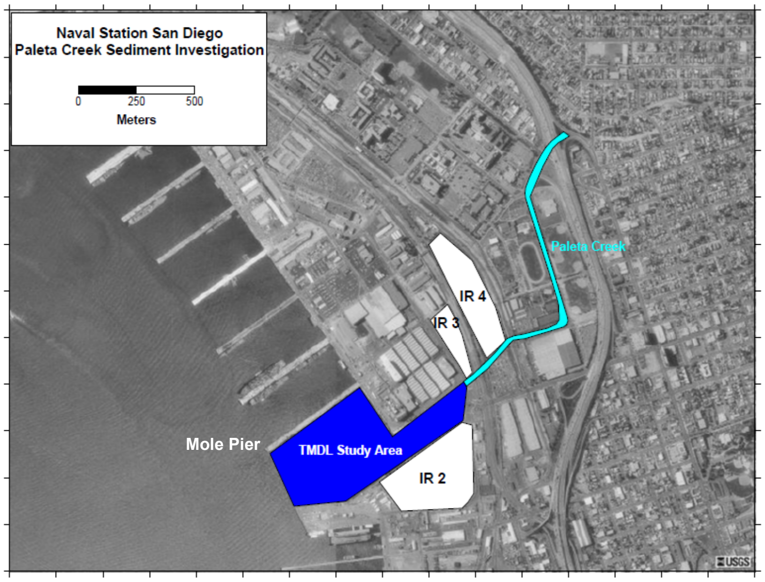


Figure C-6. Area for Total Maximum Daily Load (TMDL) study including Paleta Creek, and Mole Pier in San Diego Bay.

A tiered approach was followed in sampling. Rapid Sediment Characterization (RSC) techniques were used for metal measurement in over seventy locations using a field portable XRF, and these data were used in the selection of fifteen locations for full laboratory analyses. Measured metals by XRF include Cu, Zn and Pb, and the full laboratory analyses include some additional metals, PAHs, PCBs, and other ancillary data including grain size.

Analysis of metal concentration distribution gradients in sediments indicates different sources to the area of study. Concentration gradients of Cu show higher concentrations in the middle of the TMDL area of study (yellow area in Figure C-7, Cu), indicating possible sources within that area. In contrast, concentration gradients of Zn and Pb indicate sources from Paleta Creek (orange color in Figure C-7, Zn, orange and red in Figure C-7, Pb). Therefore, mitigation efforts may be required upstream from the TMDL area, and identification of sources is required for those mitigation efforts.

Information on sources of some metals can also be derived from bivariate metal plots. An example of this is shown in Figure C-8, for the comparison between sediment concentrations of Pb (y-axis) vs. Cu (x-axis). In this plot, the data from sediments collected by the pier area show a single trend with a large range in Cu concentrations, and relatively lower Pb concentrations. In contrast, sediments collected upstream in Paleta Creek indicate greater range of Pb concentrations, while having a narrower range of Cu

concentrations. This could be an indication of Cu sources within the Pier Area, and Pb sources from upstream Paleta Creek.

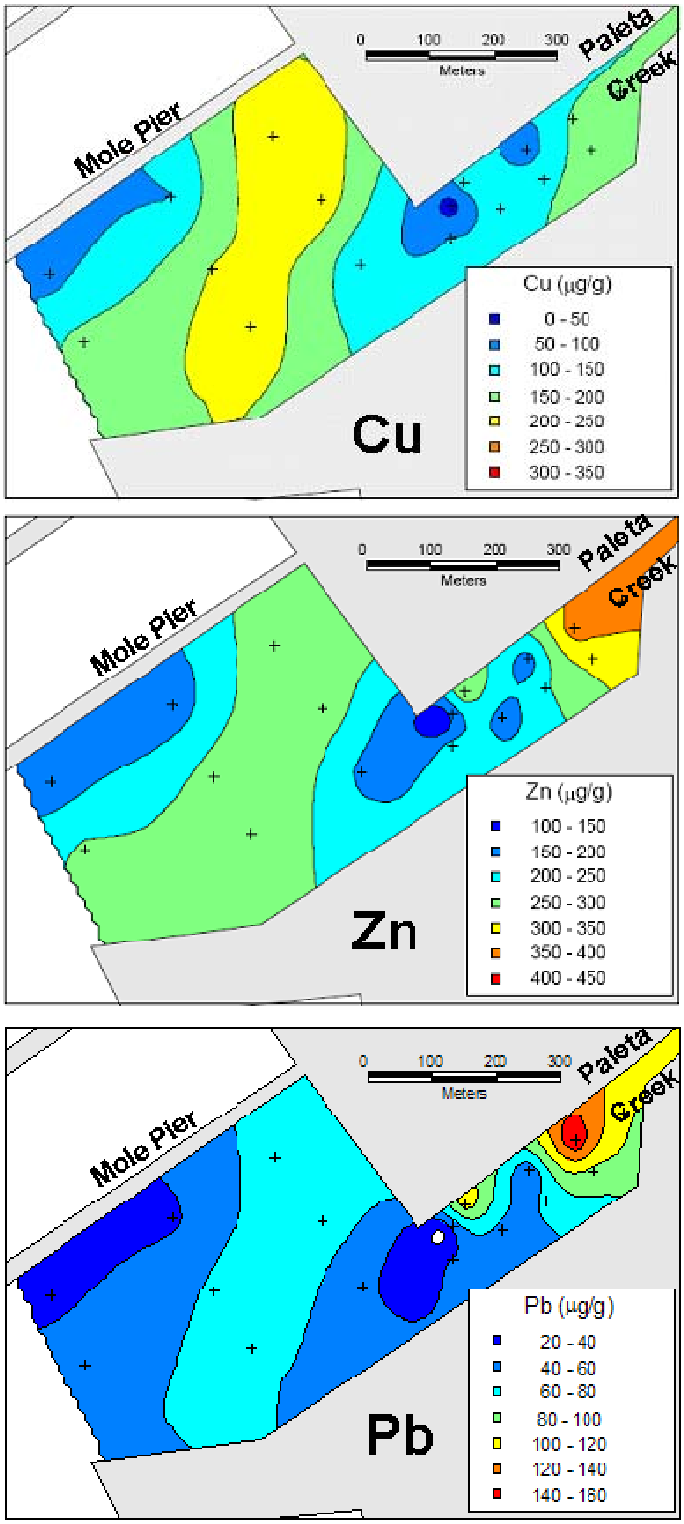


Figure C-7. Sediment concentration gradients (µg/g; micrograms per gram) of Cu (top), Zn (center), and Pb (bottom) in the Paleta Creek TMDL area.

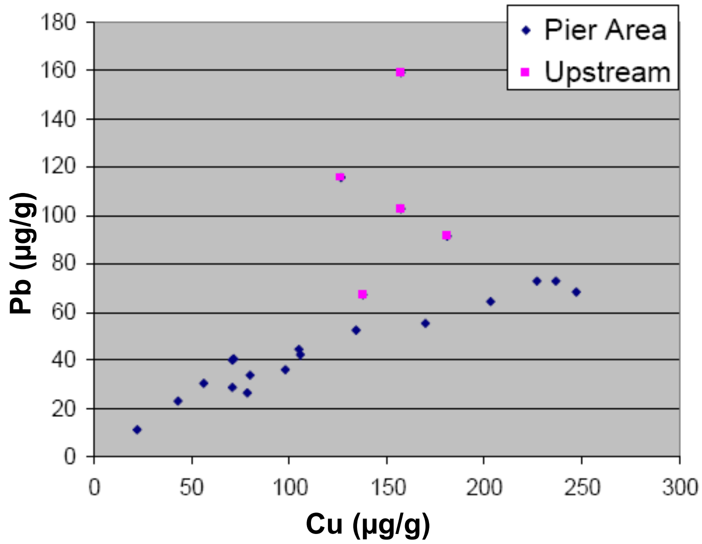


Figure C-8. Concentrations (µg/g) of Pb vs. Cu in sediments of the Paleta Creek TMDL area.

As illustrated in the case for metal gradients in surface waters, statistical analysis can also be used to differentiate sources for sediment data. Analysis of a suite of nine different metals in Paleta Creek by PCA indicated distinctive differences between sources from the pier and upstream areas, but this analysis also included definition of areas not affected by these sources, defined as reference sites (Figure C-9). Extending the PCA analysis to include data from the creek resulted in identifying two potential source sites in the creek (red circles labeled A and B in Figure C-10).

The significance of these two potential sources of Pb can be explained using stable isotopic ratios. All metals have at least one isotope, some have several, and ICP-MS is able to measure the concentration of each isotope separately, instead of measuring the total concentration of the metal. A plot of the ratios of the different isotopes can be used to differentiate sources, and to quantify the effect of each source. Split samples from the Paleta Creek TMDL study were analyzed for Pb isotopes (i.e., Pb^{206}, Pb^{207} and Pb^{208}) by ICP-MS. Figure C-11 is the plot of Pb^{208} / Pb^{206} vs. Pb^{207} / Pb^{206} ratios for the sediment samples, and clearly indicates that Source A has a pronounced effect on the other sediment samples. In comparison, source B is more closely related to ambient sediment concentrations within the area of study.

In this example, the use of isotopic ratios was used to differentiate between two potential sources in a relatively small area of study. Similar approach has been used for differentiation of metal sources in extremely extensive areas (i.e., Pacific central and coastal waters; Flegal *et al*., 1984, 1989). The example illustrates the potential of isotopic ratios for fingerprinting sources of metals.

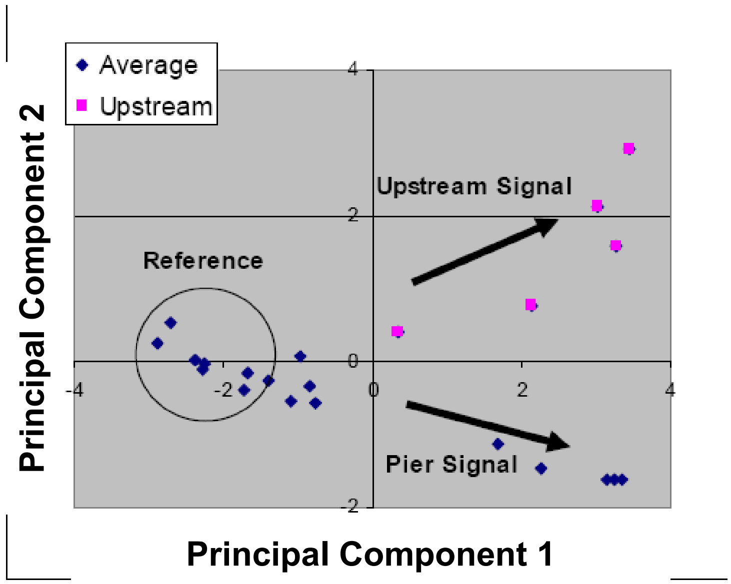


Figure C-9. Principal Component Analysis of a suite of metal concentrations in sediments from Paleta Creek TMDL study area.

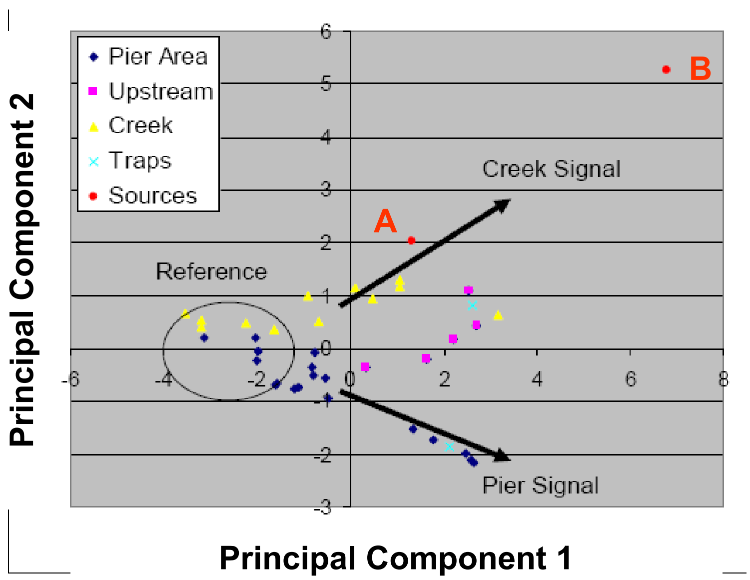


Figure C-10. Principal Component Analysis of a suite of metal concentrations in sediments from Paleta Creek TMDL study area; but extended to consider data from Paleta Creek.

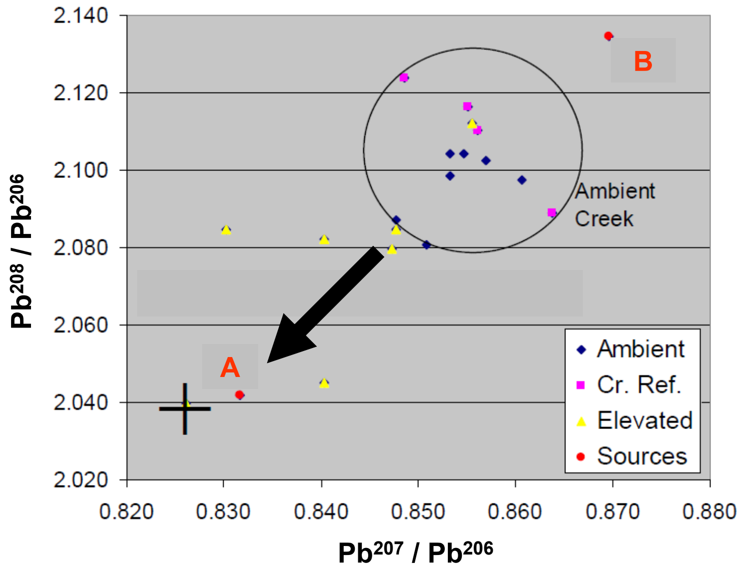


Figure C-11. Plot of lead isotopic ratios for sediments from Paleta Creek TMDL study area.

<u>Ratios of Stable Isotopes in Waters and Sediments</u>. Initial measurements of copper isotopic ratios in San Diego Bay and other DoD harbors was supported by SERDP thru project CP-1158 on *Speciation, Sources and Bioavailability of Copper and Zinc in DoD-Impacted Harbors and Estuaries*. This project, leaded by Martin Shafer at the University of Wisconsin in Madison, included the objective of determining if sources of Cu in harbors are amenable for identification by unique stable isotopic signatures. The task was not completed within the time framework of Project CP-1158, as it required "*some quite recalcitrant analytical hurdles (to) demonstrate the technical feasibility of measuring extremely small stable isotopic ratio variations of copper in some very nasty matrices.*" Identification of copper sources is an important subject to the DoD, and the Navy Environmental Sustainability Development to Integration (NESDI) program supported an effort on completing this task. Results for this latter effort are included here.

Isotopic ratios of stable isotopes of the metal of interest can be used for source identification and apportionment in complex situations. This technique is based on small differences in the ratios of the stable isotopes (i.e., non-radiogenic or isotopes that do not decay radioactively) of a given metal; ratios that are fixed at the moment of the formation of the geologic ore from which the metal is extracted (Johnson, 2004; Zhu *et al*., 2002). Therefore, by means of determining the isotopic ratios of the original ores or refined materials, and/or defined contributing end-member sources in a specific environment, and by comparison with isotopic ratios determined in field/environmental samples it is possible to differentiate and to estimate the contribution from several sources of the metal. Novel analytical techniques have been developed that enable the fingerprinting and tracking of copper using measured variations in ratios of its two stable isotopes (^{65}Cu and ^{63}Cu; Li *et al*., 2009; Moynier *et al*., 2007; Bermin *et al*., 2006; Zhu *et al*. 2000; Maréchal *et al*., 1999).

The viability of stable isotopic ratios as source reconciliation tracers requires that, at a minimum, two basic tenets are satisfied: (a) that the various ores or sources of concern

have different isotopic ratios (fingerprints), and (b) that geological, biological or environmental processes, on relevant timescales, do not change these source profiles. The first criterion is required in order to differentiate the sources, and it has been confirmed, in a general sense, as significant variation of over 9 per mil of copper stable isotope ratios in potential ore sources and environmental reservoirs (Li *et al*., 2009; Markl *et al*., 2006; Chapman *et al*., 2006; Zhu *et al*. 2002, 2000; Gale *et al*., 1999; Maréchal *et al*. 1999). The second criterion assures that the measured isotopic ratio reflects sources, and not environmental processing of the source materials. Isotope ratios in many systems, particularly the light isotopes, (hydrogen, carbon, nitrogen, oxygen and sulfur) are significantly impacted by mass-dependent environmental (physical and biological) processing, and in-fact this fractionation is the basis of several important environmental forensics tools. Even for transition metals, mass-dependent isotopic fractionation during low-temperature environmental processing has been observed (e.g. Fe), though the fractionation is quite small. In the context of copper source fingerprinting isotopic fractionation of copper during environmental release/transport is considered a minor process within a relatively small defined geographical region and over decadal time-scales, though substantial additional research is required to definitively confirm this working premise.

In addition to these considerations, there is also the challenge of precise measurement of very small variations in isotopic ratio (less than a few per mil). Each copper isotope have a very narrow range in concentration, and state-of-the-art instruments are required to measure isotopic signals with enough precision to discern these ranges. The only instrument available to measure these ranges is the multicollector, magnetic sector, inductively coupled plasma mass spectrometer (MCMS-ICP-MS; Shafer *et al*., 2005). The MCMS-ICP-MS is a research grade instrument, not commonly found in contract analytical laboratories, which can produce remarkably precise isotope ratio data, in certain systems better than 0.0001% (10 ppm, 0.01 per mil, 0.01 ‰), a level of precision that is more than adequate to accurately quantify stable isotopic fractionation of stable (i.e., non-radiogenic) element systems.

During the CP-1158 effort, the Shafer team was able to determine some isotopic ratios for a suite of potential sources to DoD harbors (Figure C-12). The results from the Shafer team show that Cu ores, Cu salts, Cu metal, and antifouling coatings (AFC) have a tendency for del ^{65}Cu values (delta ^{65}Cu; ratio of measured ^{65}Cu in sample to ^{65}Cu in High Purity Standard) relatively close to 0.0. In contrast, seawater samples from both Norfolk Harbor and San Diego Bay have positive del ^{65}Cu values in the range between 1.0 to more than 2.5. These results support the suggestion that there are other Cu sources in these harbors, with a “larger” del ^{65}Cu (green oval in Figure C-12). The results from CP-1158 and those from other researchers (Bermin *et al*., 2006; Hull *et al*., 2008; Petit *et al*., 2008) supported a NESDI effort to expand the database of Cu sources in San Diego Bay. In this new effort, a broad range of samples were evaluated for their stable Cu isotopic composition, including Cu-ablative paint and extracts, and San Diego Bay water and sediment from selected sites where runoff to the Bay occurs.

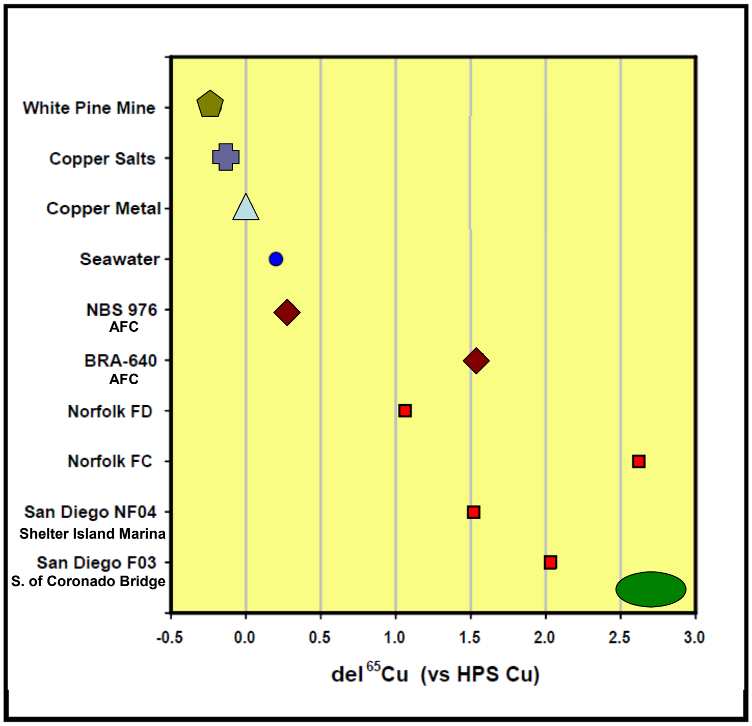


Figure C-12. Copper isotopic ratios measured during the CP-1158 effort from a suite of sources (modified from Shafer *et al*., 2005). The sources include ores (White Pine Mine, pentagon), salts (grey cross), copper metal High Purity Standard (HPS Cu; light blue triangle), leacheates from antifouling coatings (AFC; NBS-976, BRA-640; dark red diamonds), and seawater from Norfolk Harbor and San Diego Bay (red squares). The isotope ratio (del = delta) is given as the ratio between ^{65}Cu and HPS Cu. The green oval shows the isotopic ratio expected from other sources within San Diego Bay.

As indicated for CP-1158, measurement of isotopic ratios in the new suite of samples examined during the NESDI effort required extensive chemical manipulation. This was required to reduce the impact of the matrix of salt water or sediments on the measurements. The chemical processing resulted in a consistent final matrix of 2% nitric acid for all samples for MCMS-ICP-MS analysis.

The new measurements indicate that the trend of isotopic ratios in seawater samples from San Diego Bay is the combined influence of anthropogenic and natural geologic sources (Figure C-13). The results from this more extensive analysis of samples from San Diego Bay show that the only samples with a del^{65}Cu more positive than those from seawater samples are those from sandy sediments with low copper concentration, that are considered reference sites, and are representative of geologic sources of copper to the Bay. This is also supported by the fact that the Standard Reference Material (SRM) Basalt Hawaii Volcanic Observatory (BHVO) also measured del^{65}Cu more positive that the rest of the samples (Figure C-13). In contrast, del^{65}Cu values closer to zero, and lower than those measured in Bay seawater, are representative of anthropogenic sources, e.g. from antifouling coatings (AFC) and silty sediments with larger copper concentrations.

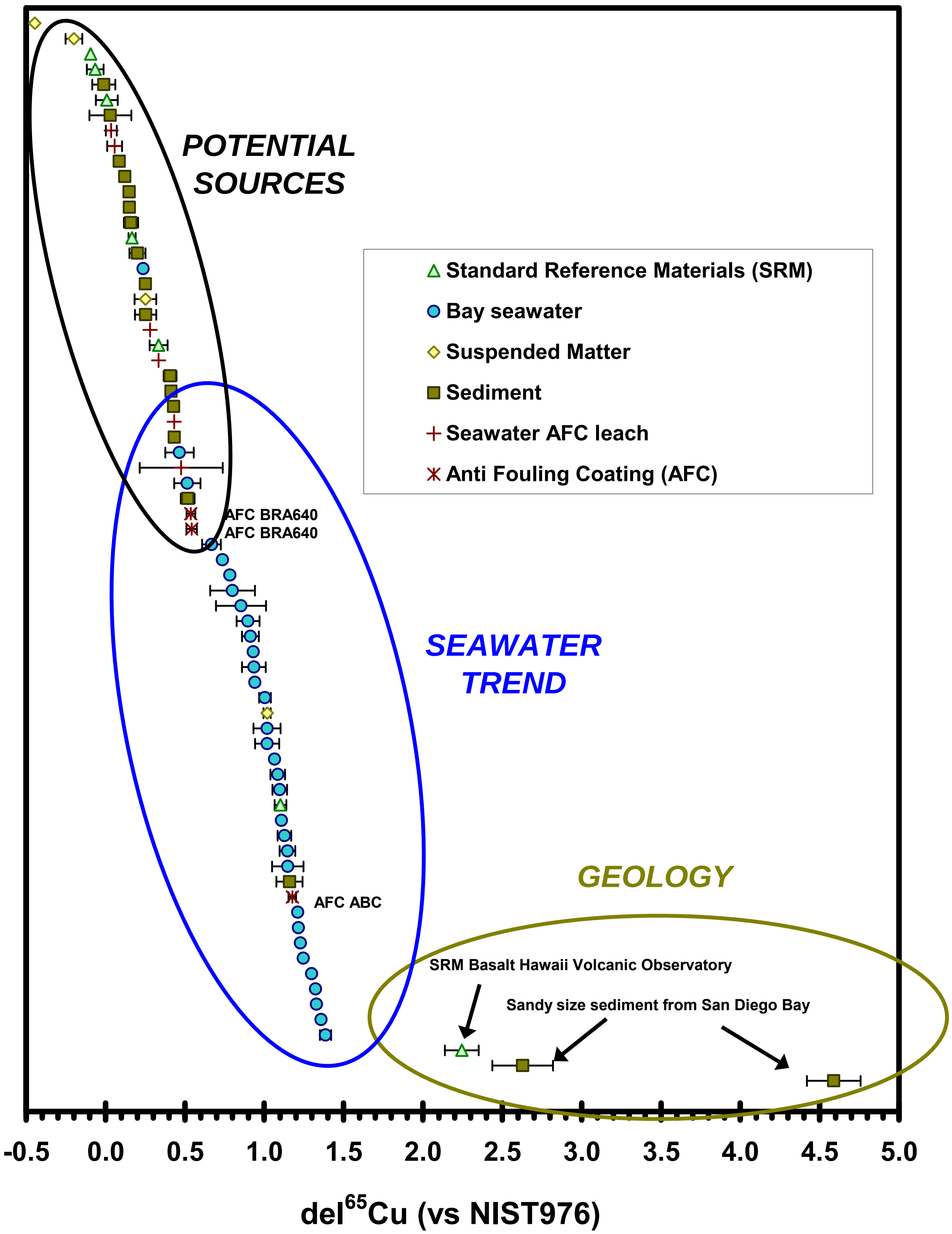


Figure C-13. Copper isotopic ratios measured for a suite of samples from San Diego Bay. The isotopic ratio is given as the del^{65}Cu vs. SRM NIST976, Isotopic Standard for Copper. The apparent distinction in isotopic ratios illustrates the effect of the geology and anthropogenic sources on the distribution of copper isotopic ratios in the waters of San Diego Bay.

It is important to note that the samples analyzed for isotopic fingerprinting of sources, as presented in previous pages, were samples collected for a variety of different efforts. Each of these efforts was focused on answering different technical or regulatory questions, but was not focused specifically on sourcing assessment. This caveat precludes a more detailed evaluation of sources within San Diego Bay using the existing sample data, but such an evaluation could be accomplished with a dedicated sampling and analysis effort directed at source assessment.

Appendix C References

Bermin J., D. Vance, C. Archer, and P.J. Statham. 2006. The determination of the isotopic composition of Cu and Zn in seawater. Chemical Geology 226:280-297.

Bruland, K. W.; Coale, K. H.; Mart, L. 1985. Analysis of seawater for dissolved cadmium, copper and lead: An intercomparison of voltammetric and atomic absorption methods. Marine Chemistry 17: 285-300.

Chadwick, D.B., I. Rivera-Duarte, A. Carlson, G. Rosen, L. Kear-Padilla, A. Zirino, T. Boyd, J. Gieskes, and O. Holm-Hansen. 2005. Determining the Fate and Ecological Effects of Copper and Zinc Loading in Estuarine Environments: A Multi-Disciplinary Program. Final Report to SERDP Project CP-1156, http://www.serdp.org/Research/upload/CP_1156_FR.pdf, 104pp.

Chapman J.B., T.F.D. Mason, D.J. Weiss, B.J. Coles, and J.J. Wilkinson. 2006. Chemical separation and isotopic variations of Cu and Zn from five geological reference materials. Geostandards and Geoanalytical Research 30:5-16.

Flegal, A.R., T.F. Duda, and S. Niemeyer. 1989. High gradients of lead isotopic composition in north-east Pacific upwelling filaments. Nature 339(6224): 458-460.

Flegal, A.R., B.K. Schaule, and C.C. Patterson. 1984. Stable isotopic ratios of lead in surface waters of the Central Pacific. Marine Chemistry, 14: 281-287.

Gale, N.H., A.P. Woodhead, Z.A. Stos-Gale, A. Walder, and I. Bowen. 1999. Natural variations detected in the isotopic composition of copper: possible applications to archaeology and geochemistry. International Journal of Mass Spectrometry, 184(1):1-9.

Hull, S., M. Fayek, F.J. Mathien, P. Shelley, and K.R. Durand. 2008. A new approach to determining the geological provenance of turquoise artifacts using hydrogen and copper stable isotopes. Journal of Archaeological Science 35:1355-1369.

Katz, C.N. 2005. Sediment Assessment Study for the Mouths of Chollas and Paleta Creek, San Diego. Phase I Final Report to San Diego Regional Water Quality Control Board and Commander Navy Region Southwest, 200pp.

Li, W., S.E. Jackson, N.J. Pearson, O. Alard, and B.W. Chappell. 2009. The Cu isotopic signature of granites from the Lachlan Fold Belt, SE Australia. Chemical Geology 258:38-49.

Maréchal, C.N., P. Télouk, and F. Albarède. 1999. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chemical Geology, 156:251-273.

Markl, G., Y. Lahaye, and G. Schwinn. 2006. Copper isotopes as monitors of redox processes in hydrothermal mineralization. Geochimica et Cosmochimica Acta 70:4215-4228.

Moynier, F., J. Blichert-Toft, P. Telouk, J.-M. Luck, and F. Albarede. 2007. Comparative stable isotope geochemistry of Ni, Cu, Zn, and Fe in chondrites and iron meteorites. Geochimica et Cosmochimica Acta 71:4365-4379.

Petit, Jérome, et. al. 2008. Development of Cu and Zn isotope MC-ICP-MS measurements: application to suspended particulate matter and sediments from the Scheldt Estuary. Geostandards and Geoanalytical Research. 32, 149-166.

Shafer, M., D. Tang, J. Hemming, B. Beard, and D. Armstrong. 2005. Speciation, Sources and Bioavailability of Copper and Zinc in DoD-Impacted Harbors and Estuaries. Final Report to SERDP Project CP-1158.

Zhu, X.K., R.K. O'Nions, Y. Guo, N.S. Belshaw, and D. Rickard. 2000. Determination of natural Cu-isotope variation by plasma-source mass spectrometry: implications for use as geochemical tracers. Chemical Geology, 163:139-149.

Zhu, X.K., Y. Guo, R.J.P. Williams, R.K. O'Nions, A. Matthews, N.S. Belshaw, G.W. Canters, E.C. de Waal, U. Weser, B.K. Burgess, and B. Salvato. 2002. Mass fractionation processes of transition metal isotopes. Earth and Planetary Science Letters, 200:47-62.

APPENDIX D

Case Study on Pollutant Source Tracking for Organics (PCBs)

ESTCP ER0826 Case Study Report for the Pollutant Source Tracking (PST) Project

Integrated Forensics Approach to Fingerprint PCB Sources using RSC and ACF

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SPAWAR Systems Center Pacific

Introduction

As part of the Pollutant Source Tracking project under the Navy's NESDI program, this case study was developed to demonstrate PCB fingerprinting techniques at a Navy sediment site. It utilizes data and information generated under the ESTCP program by project ER0826 titled "Integrated Forensics Approach to Fingerprint PCB Sources using Rapid Sediment Characterization (RSC) and Advanced Chemical Fingerprinting (ACF)". The format of this case study report will follow the format of a 12/03/09 presentation given at the SERDP/ESTCP annual meeting and will actually use the slides from that meeting's presentation as figures for this report. Much of the background for the development of this project is discussed in the original proposal, and additional details about many of the topics in this case study report can be found in documents available under this project's name and number on the ESTCP website. The format of this case study report will include some introductory information about forensic approaches first, followed by the technical approach developed for this project, and finally some discussion of the ways to transfer forensics data into useful information to stakeholders from various technical backgrounds.

Sediments are often considered the ultimate sink for contaminants in aquatic settings. Once in the sediments, however, contaminants may be reintroduced into the overlying water column or the biological community by a number of physical, chemical, and/or biological processes. Sediment contaminants therefore remain a regulatory concern and are often the subject of expensive regulatory actions. Determining the original source of contamination to a heterogeneous matrix such as sediments is a requirement for both Clean-up and Compliance programs within the military. Understanding the source of contaminants to sediment in industrial settings is a prerequisite to implementing any proposed sediment remedial options under the Clean-up program. This is due to the fact that the sources must be controlled prior to remedial efforts to ensure that recontamination can be avoided. An additional reason for source identification includes ensuring that costs of any remedial efforts can be fairly allocated among multiple principle responsible parties (PRPs). In some instances, elevated levels of polychlorinated biphenyls (PCBs) in sediment have led to impairment designations requiring the development of total maximum daily loads (TMDLs) and subsequent waste load allocations under Compliance programs. Because of this, development of site-specific forensic investigations and TMDLs are closely linked. The need to develop these types of TMDLs also requires the development and use of a forensics approach to fingerprint contaminant sources so that potential load reductions can be allocated. Without a forensics study the standard approach is to assume the most visible, nearby facility is the source of contamination, and this often turns out to be a military facility. The forensics approach to be demonstrated in this project includes two primary components: 1) rapid sediment characterization (RSC) technologies that provide for wide spatial and temporal coverage to delineate sediment contaminant heterogeneity and semi-quantitative characterization in a cost effective manner; and 2) advanced chemical fingerprinting (ACF) on a selected subset of samples to provide congener analyses that will delineate sources. ACF includes both advanced laboratory chemical analysis of samples, and the

application of sophisticated data analysis and interpretation methods to determine the number of sources and their relative contributions around the site.

Slides 1 through 3 provide the introductory information concerning the ESTCP project and lay out the general outline for what will be discussed in this case study report. Slide 1 presents the title and ESTCP project number for this project, and additional information can be obtained from the ESTCP website with this information. Slide 2 provides the general outline for the format of this report. Following a brief introduction to the project, there will be a general discussion of environmental forensics to provide the reader with an idea of what forensics projects are attempting to accomplish. The main portion of this report will lay out the six step approach to conduct a forensics study, using our first demo site (Hunters Point Shipyard (HPS)) as a case study. Included in this section will be a discussion of the mechanics (how we work through each step in the process) with some metrics that are used to access the performance of the techniques. The report will finish by showing some techniques to visualize the data and provide useful information to the general audience. While those with experience in forensics studies may appreciate the mechanics in various steps of the approach (including metrics used to quantitatively evaluate the performance of various techniques), those with more of a general interest in forensic studies may find the later section more informative when the spatial relationships of various potential end member sources are discussed. In fact, although it is generally known that forensics studies need experienced personnel working with high quality data to be successful, an often overlooked requirement for success is a way to transfer the information at multiple technical levels to ensure utility to a broad audience with a range in technical backgrounds. To help accomplish this important task, the first prerequisite is to assemble a team with a varied background to bring a range of experience to the project. As an example, for this ESTCP project we have assembled the project team shown in Slide 3. Leather at SPAWAR will act as overall lead on the project and oversee all the immunoassays (RSC) that will be used in the contour mapping. Durell at Battelle will provide all of the congener analyses (part 1 of ACF) that will be used to actually fingerprint the PCB sources, and he will work with Johnson at University of Utah on statistical analyses of the data (part 2 of ACF). All of the data used in this study are leveraged from regulatory projects to help reduce the costs of the ESTCP project, so Forman at the Navy and Mills at the EPA are responsible for providing all the data at the from existing regulatory projects. This provides an example of how leveraging efforts with existing regulatory projects to pay for the expensive field and analytical costs can allow forensics studies to be done in a more cost-effective manner.

The novelty of the ESTCP project is to demonstrate an integrated approach to characterize PCB contamination that combines sediment screening technologies on a large number of field samples followed by detailed PCB congener analysis in conjunction with advanced chemical fingerprinting data interpretation on a subset of selected laboratory samples to identify sources. The idea of combining RSC with ACF is to cost-effectively maximize the benefits of each method while at the same time offsetting the limitations of each method. For example, RSC provides a cost-effective technique for spatial (and perhaps temporal with core data) coverage, allowing chemical gradients to be determined for initial indications of potential sources. However, the immunoassays used

for RSC only provide total Aroclor PCB data and do not allow individual congeners to be determined that are required for actually fingerprinting sources. ACF normally requires specialty analyses beyond the scope of normal regulatory requirements and therefore also at higher costs than many standard regulatory analyses. Many regulatory programs only require that the concentrations for total Aroclors or maybe 18 major congeners be determined and reported. However, ACF for PCBs requires that approximately 50-100 PCB congeners be determined so that multiple source and alteration patterns can be differentiated. Therefore an integrated forensics approach can be designed in a cost-effective manner using a combination of RSC and ACF techniques. A large number of lower cost RSC samples (to suggest "where" sources are present) can be used to map concentration gradients and visualize source areas to allow for a more representative sampling of fewer higher cost ACF samples (to confirm "what" sources are present) in an integrated forensics approach.

PCB Forensics Background

Polychlorinated biphenyls (PCBs) are not a single compound, but a group of 209 individual chemical compounds (termed congeners) depending on where the chlorines are placed on the biphenyl molecule. Slide 4 shows a figure of the biphenyl molecule and the numbered locations where chlorines may be located. In the United States, Monsanto produced commercial mixtures of congeners (termed Aroclors) for specific applications from the 1930s to 1970s. Also shown on Slide 4 are several bar chart "fingerprints" for standard Aroclors including Aroclor 1254 (with 54% chlorine) and Aroclor 1260 (with 60% chlorine). These fingerprints show the proportion (as %) of the common 18 NOAA Status and Trends congeners, and later we will show some more complex bar chart fingerprints with 50 to 100 congeners. It is easy to see the difference between these two fingerprints of standard Aroclors even by eye, since Aroclor 1254 has more of the middle weight congeners and Aroclor 1260 has more of the heavy weight congeners. But most "real" sediment sample fingerprints can contain a mix of around two to five Aroclor sources, and there may be additional alteration (including solubilization, dechlorination, etc.) that tends to obscure the original Aroclor patterns. The bottom bar chart fingerprint is a simple 50%/50% mix of Aroclor 1254 and Aroclor 1260, which becomes harder to differentiate by eye so it is easy to see why more complicated statistical analyses are required to "unmix" the original sources from these real PCB sediment fingerprints.

Slide 5 is a word slide that provides the basic idea of what forensics studies are trying to accomplish. To determine the sources of PCB contamination in sediments at a site, we usually are working at sites with large datasets of PCB congener analyses. We therefore use some form of multivariate statistical analyses (such as principal component analysis (PCA)) to obtain three parameters: 1) the number of sources; 2) the congener composition (bar chart fingerprint from previous slide) of each source; and 3) the proportion of each source in each sample around the site. These types of studies looking for PCB sources in sediments have been done for the past decade or so, and many of the techniques are based on work EPA has been doing for over 25 years for air pollution source fingerprinting. Slide 6 provides a picture slide of how this is done, where the matrix algebra equation at the bottom of the slide is shown as a cartoon. Many of the

statistical analyses used in forensics start with some form of PCA matrix decomposition, where we start with a large matrix of I sample rows and J parameter (congener) columns. This large dataset is decomposed into source matrix Y and a loading matrix X to find the three parameters listed above. The “trick” is to obtain a reduced set of sources (N is actually the number of principal components, but in this highly simplified example it can be thought of as the number of sources) from the larger number of samples and congeners (N << I or J, which may represent around 1 to 5 sources obtained from around 20 to 100 samples and congeners).

Technical Approach

The DoD “problem” that requires a fingerprinting solution is depicted in Slide 7. Since the military is often the most visible stakeholder on an industrial waterfront, they are often pointed at as the source for all sediment contamination problems even though other industries may be contributing contamination. This has long been a problem for sediment cleanup sites, but more recent compliance issues such as TMDLs have also identified PCBs as a major problem. Both Cleanup and Compliance programs could therefore use a technically defensible method to fingerprint sources to not only ensure shutoff of any continuing sources, but also to help apportion TMDL load reductions and/or cleanup costs.

The proposed integrated forensics approach is summarized in Slide 8. To determine the sources of sediment PCBs, we combine Rapid Sediment Characterization (RSC) and Advanced Chemical Fingerprinting (ACF). RSC may include techniques such as immunoassays to map PCB concentration gradients to provide insight into potential locations of sources. Then a small subset of samples can be run for ACF (GC/MS congener analysis) to determine characteristic fingerprints to identify sources. For example, the older surface sediment contour map from the first demo site shows two potential source areas, one to the east by the former landfill and one to the west by the creek. If the red sample points from the contour map had a composition represented by the red bar chart fingerprint, this might represent the source fingerprint from the landfill. And if the green sample points over by the creek had a composition represented by the green bar chart fingerprint, this might represent the source fingerprint from the creek area (for this example ignore the mapped green sample locations next to the red locations by the landfill since the color coding for the contour map was done years before the bar charts). Then all of the blue sample locations would have fingerprints like the blue bar chart fingerprint, and the fingerprinting study would have to determine how these two sources were mixed out into the embayment to give different blue bar chart fingerprints for each sample location.

The actual analytical technologies used in the demonstration are shown on slides 9 and 10. Since this is an ESTCP project to demo widely acceptable technologies, for RSC commercially available immunoassay techniques were used. These types of immunoassay techniques were first developed for the medical industry and adapted for use in the environmental field. We will employ modified EPA Method 4020 for immunoassay use in sediments. For ACF, modified EPA Methods 680/1668a will be used to detect a large

number of diagnostic congeners. The laboratory data are then input into advanced statistical analysis methods to determine the number of sources and their relative contributions around the site. The figure in Slide 10 shows a comparison of two fingerprints (bar charts of percent contribution of long list of 50 congeners). The top fingerprint (a) was generated from one of these statistical analysis methods and represents a predicted endmember source candidate for a site. The lower fingerprint (b) is from a laboratory analysis of an Aroclor 1242, and represents a proposed match to the top fingerprint. A visual comparison of top and bottom bar charts shows similar congener distributions, suggesting that these fingerprints match and Aroclor 1242 is indeed the endmember source in this example. To provide a more objective means for comparison, quantitative metrics can be used to evaluate the fit (this cosine theta metric will be discussed later). Like the correlation coefficient (R squared values) which compares correlation of two variables, the cosine theta metric compares the correlation of two matricies of variables (like the bar chart fingerprints in the figure), and the scale used of these metrics is the same so values closer to 1.0 indicate better correlation.

The combined use of RSC and ACF are only two steps in the overall forensics approach. The overall sequence of steps, or tasks, that will be employed include (1) evaluation of the site's potential as a forensics study site, (2) development of a conceptual site model, (3) development of a defensible study design, (4) demonstration of rapid sediment characterization (RSC) screening, (5) demonstration of advanced chemical fingerprinting (ACF), and, finally, (6) synthesis and presentation of the results in a final report. This process is shown in Slide 11, and the following slides will show how it has been applied at our first demonstration site. Slide 12 shows the first step of selecting the site for the forensics demonstration. Hunters Point Shipyard is a Navy BRAC (Base Realignment and Closure) site in San Francisco Bay just south of the city of San Francisco. Due to the regulatory Remedial Investigation (RI) and Feasibility Study (FS), there is a large amount of PCB data available to leverage into this project. The area of interest is the South Basin, where there appears to be high PCB gradients associated with a former landfill at the northeast side of the triangular embayment, and a second PCB source area at the mouth of Yosemite Creek at the west side of the embayment. Both SPAWAR and Battelle were involved in the FS, and were actually involved in developing a forensics study along the same lines as outlined in this project. Ultimately, the regulatory project decided to move forward with their FS without completing the forensic study so all these data become available to leverage into this demonstration project. The regulatory project progressed through collecting the ACF data in Step 5, but stopped short of actually analyzing the data. This site can therefore represent the case where a large amount of pre-existing data are available, and must be evaluated to determine how much (if any) additional data are needed for a forensics study. The demonstration must then complete the steps in the forensics approach and show how other sites could work through the process by starting at any step in the process.

Slide 13 shows the second step of developing the conceptual site model (CSM). This is normally done as part of the third step of developing the sample and analysis plan, but is highlighted here by breaking it out as its own second step. The CSM should bring together all the available information at the site to allow specific testable hypotheses to be

developed. This should lead to development of specific forensic questions that can be answered with data collected as part of a sample and analysis plan developed in the next step. The figures in Slide 13 show the regulatory CSM flowchart used by the FS to track PCB movement from proposed sources on the left to receptors on the right. For the forensics CSM, data are often put into a principle component analysis (PCA) plot to visualize congener compositional variations around the site. Principal components are plotted on each axis (labeled Factor1 and Factor2 in this figure), and the samples that plot closer together represent more similar congener patterns. For example, those samples plotting closer to the bottom of the figure have congener compositions similar to Aroclor 1260, and spatially these samples are all from the northeast side of the embayment near the former landfill. And those that plot at the top of the figure have congener compositions showing a mixture of Aroclor 1254/1260 and are located on the west side of the embayment near the creek. These data come from older studies at the site where only 18 congeners are available from surface sampling, so confounding factors such as alteration (solubilization, dechlorination, etc.) are hard to address. From the limited data in this figure there appears to be two source areas (one to the northeast by the landfill and one to the west by the creek), so the forensics study should address if there appear to be any other sources (or alteration patterns) and how all these proposed sources are mixed out into the embayment in all the other samples collected around the site.

Slide 14 shows Step 3 development of a sampling and analysis plan, which under the ESTCP program is done as part of the Site Demonstration Plan. This figure shows the sampling map for the FS, with high density sampling near the northeast landfill and west creek. There is also a high density sampling between these areas to access the mixing between these two proposed source areas. Out farther in the embayment the sampling density is lower where concentration gradients are expected to be lower, but there is still interest in how the various sources are mixing out in the embayment. Since the sample and analysis plan was already completed for the FS, it was included as an appendix in the ESTCP Demonstration Plan and most of the Demonstration Plan dealt with the performance objectives that can be used to gauge the success of the project.

Slide 15 summarizes Step 4 on how the RSC data can be visualized using contour maps to help select representative samples for ACF. This figure shows a single depth horizon where variograms (plots of PCB concentration versus distance) are used by a contouring program to interpolate concentrations across the site. Usually this contouring process involves development of variograms and selection of specific contouring "rules" that will be used to generate the contour maps. As a general rule, a minimum requirement would be to include sample locations on maps to help judge how representative these contours are over sampled and unsampled areas. It is sometimes also useful to map the error terms associated with these contours to evaluate how representative these contours actually are over different areas of the site. The greater data density allowed with the lower cost RSC technique often provides sufficient coverage both horizontally and vertically to support 3-dimensional (3D) contour mapping. The main objective for this type of visualization tool is to suggest where source areas may be located and help select a representative number of samples for ACF. Since there are sufficient core data here to provide adequate horizontal and vertical coverage, we have used EarthVision software to show three

dimensional contours and volumes of sediment at various concentration ranges to highlight potential source areas and to aid in selection of ACF samples. So figures 16-20 show 3D contour maps which highlight different concentration ranges. Figure 16 shows a three dimensional block diagram of the core locations sampled for the RSC that will be used in this forensics demonstration. The front of the block shows an east-west vertical cross-section down to 5 feet, and the top of the block shows the map view of the site with the shoreline outlined by the wavy white line. Each core shows the seven sampled horizons with color coded boxes that represent concentration. The northeast side of the triangular embayment shows a large number of red boxes indicating PCB concentrations above 2000 ppb near the former landfill. The west side of the embayment where the creek enters also shows a large number of red boxes, indicating another potential source area with concentrations above 2000 ppb. Slide 17 shows the contoured volume of sediment above 2000 ppb, and the two high concentration areas are clearly seen with a thin "neck" of mixing sediment between them. Slide 18 shows the contour of sediment above 1000 ppb, indicating PCBs mixing down toward the southeast from both potential high concentration source areas as well as more mixing between the two areas leading to a thicker "neck". Slide 19 continues these trends for sediments contoured above 700 ppb. Slide 20 shows that mixing finally occurs out into the rest of the embayment above 200 ppb, and it should be noted that there do not appear to be any other source areas with comparable magnitude around the margins of the embayment. The main point of these contour maps is to provide a visualization of the RSC data to help select samples for ACF. Multiple ACF samples should be selected from each high concentration potential source area to delineate a source signature. Samples should also be taken in the mixing area between these two potential source areas to define the degree of mixing. Samples should also be taken farther out into the embayment to define the mixing of the sources out into the rest of the embayment. By stepping through these contour maps, ACF samples can be selected to cover the spatial and concentration ranges seen in the contour maps.

The performance of RSC will be assessed through the use of several metrics (additional discussion of performance objectives and metrics can be found in the Site Demo Plan). Both RSC and ACF measurements are modifications of standard EPA methods, and both can be evaluated by similar standard methods often employed by EPA laboratories. These methods include the evaluation of data quality parameters that can be characterized by five indicators of data quality referred to as the PARCC parameters: precision, accuracy, representativeness, completeness, and comparability. The specific types of quality assurance samples that can be used to evaluate precision and accuracy will vary, with additional measures being used for the other PARCC parameters. Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. Traditionally, precision of a technology is assessed with the use of field duplicate samples and the analysis of laboratory replicates. Field duplicate samples provide precision data for sample collection, field preparation, handling, and transportation procedures. Replicate sample measurements provide data for the analytical precision of the specific technology. Accuracy refers to the difference between a sample result and the reference or true value for the sample. Standard Reference Materials (SRMs) will be analyzed with each set of demonstration samples to demonstrate

accuracy. SRMs from the National Institute of Standards (NIST) or internal laboratory SRMs that have been calibrated against these NIST SRMs are generally selected to match site characteristics (PCB concentrations, Total Organic Carbon content, etc.). Alternatively, or in addition, accuracy may be determined through the analysis of laboratory control and /or field matrix samples spiked with the target analytes of interest, and the determination of the concentration and/or recovery of the target analytes. Representativeness refers to the degree to which the data accurately and precisely represent the conditions or characteristics of the parameter represented by the data. If PCB concentrations are measured at sufficient numbers around the site to allow variograms and contour maps to be generated, this parameter is typically satisfied. Completeness refers to the amount of data collected from a measurement process compared to the amount that was expected to be obtained. Obtaining high quality data with few non-detect measurements allow this parameter to be met. Comparability refers to the confidence with which one dataset can be compared to another. This is judged by looking at all the previous discussed quality assurance data among the different datasets collected at different times and measured by different laboratories. More discussion of these performance objectives, along with the actual quality assurance samples to be collected and acceptable values for these samples can be found in Chapter 5 of the Site Demonstration Plan.

Slide 21 shows an example of the performance metrics applied to the RSC data. The figure shows a raw datasheet from the immunoassay measurements, with a three point standard calibration on top to develop the relationship between PCB concentration and color change (as measured by absorbance) and a batch of twenty site samples below. The QA data is highlighted in red for measures of accuracy and precision, and as shown on this slide they pass the performance objectives laid out in the Demonstration Plan. Once the data pass these metrics, the real use for the RSC data is in building contour maps to visualize the concentration gradients around the site and allow selection of a subset of samples for ACF. Once the ACF data are obtained, comparisons to the RSC can be made as shown in Slide 22. As a measure of comparability, this type of crossplot can show a correlation coefficient (R squared) to assess fit as either fair (0.5 to 0.7), good (0.7 to 0.9), or excellent (>0.9).

After the RSC contour maps are used to select and measure the ACF samples, multiple levels of statistical analyses are possible to investigate the ACF forensics data. These various techniques use slightly different methods, but basically all generate a solution whereby multivariate sample profiles can be interpreted to generate an estimate of the original source compositions. The simplest approaches use PCB congener compositional profiles, other diagnostic ratio crossplots, or modified least squares procedures to generate mixing proportions based on an assumed source profile matrix. Such methods work best with a limited number of relatively well known sources. In contrast, more involved self-training receptor modeling methods are better suited to those situations where one cannot (or wishes not to) assume the contributing source fingerprints. These methods differ in their mathematical detail, but are similar in that they do not require *a priori* source profiles. This is in fact their strength. These methods try to minimize

assumptions, and are data-driven. These more involved methods include methods such as polytopic vector analysis (PVA), which is described in the next slide.

The Step 5 ACF Demonstration is summarized in Slide 23. The Site Demo Plan calls for the use of PARCC parameters to check the data quality in a similar fashion as that described above for the RSC, and then use a weight of evidence approach to investigate and analyze the ACF data. Simple crossplots and PCA plots can be used to visualize the congener compositional patterns. After this the more advanced statistical methods (such as the PVA method shown in the figure) can be used to select the number of endmember sources, their congener compositions, and their relative contributions to samples around the site. The figure in Slide 23 shows an early run of the HPS dataset being investigated by PVA. Like many of the advanced statistical methods, PVA starts with PCA so the points here represent the sample congener compositions plotted in three dimensional principal component space (similar to what was shown in the two dimensional PCA plot in Slide 13). A simplex, or geometric shape, is iteratively enlarged, rotated, and contracted until all sample points are contained within the geometric shape. In this case where a three endmember solution is used, a triangle is used to enclose the data points. The smaller black triangle represents the first iteration and the larger blue triangle represents the final iteration where all the sample points have been enclosed within the triangle (note there is a 5% overshoot allowed to help close the geometric shape around the datacloud so one point here still falls outside the triangle). The enclosed sample points then all represent positive linear combinations of the three end-members (EMs) which are represented by the congener compositions at the corners of the triangle. By running different groups of samples in multiple runs, we are able to reach a consistent solution with the same three EMs being obtained from the different runs. This provides us with some level of confidence that we have a robust solution not based on any special samples.

As was done with the RSC data, the next several slides will show examples of metrics used to assess the performance of the ACF data. Slide 24 shows a simple congener crossplot where the correlation coefficient is used to assess whether there is a single relationship (representing one source) or multiple relationships (representing multiple sources). If multiple sources are indicated, it must also be decided if these represent original sources or alteration patterns from processes such as solubilization, volatilization, or dechlorination. The example in Slide 24 plots PCB180 versus PCB146 for multiple horizons in core SB81. This single core shows a higher slope for the surface horizons and a lower slope for the deeper horizons. At first this greater amount of the lower chlorinated congener at depth might suggest dechlorination as an alteration process, but comparison to other congener pairs does not indicate preferential enrichment of the lighter congeners so this change in congener composition with depth is interpreted as a change in original source compostions. Although many congener pairs were viewed, these congeners are selected and plotted as an example because they show a simple ratio of 10 in Aroclor 1260 and a ratio of 1 in Aroclor 1254. So the surface horizons with slope of 9 represents an Aroclor 1260/1254 mix of approximately 90%/10%, and the deeper horizons with a slope of 5 represents an Aroclor 1260/1254 mix of approximately 50%/50%. It becomes obvious that this type of congener by congener analysis would

become tedious and time consuming, especially with datasets of 50 to 100 individual congeners. Therefore multivariate analyses such as PCA or PVA are used to look for these types of patterns in congener correlations to aid in data interpretation.

The next two metrics are used together to help assess the performance of techniques such as PVA to decide how well the modeled data fit the measured data. Slide 25 shows the use of cosine theta as a metric to compare the correlation of two matricies, in a similar fashion to how correlation coefficient is used to assess the correlation in the previous slide. In the PVA plot repeated from Slide 23, each sample's congener composition can also be represented as a unit length vector originating at the origin of the three principal component axes and terminating at the points drawn in principle component space shown in the figure. The cosine of the angle (termed cosine theta) between these vectors can be used as a metric to gauge the correlation between these two matricies, or fingerprints. Points that lay on top of each other in principal component space represent the same congener compositions and would have a zero angle between their vectors and a cosine theta value of 1.0 (perfect correlation). As sample points with differing congener compositions plot farther away from each other in principal component space, the angle between their vectors will increase and the cosine theta will decrease. As discussed in the previous slides, multivariate techniques like PVA look for correlation patterns in groups of congeners and those samples with more similar patterns plot closer together in principal component space. For example, those samples that plot over on the right side of the triangle near EM1 all show compositions with greater amounts of middle weight congeners and the EM1 composition best fits an Aroclor 1254 pattern. Those samples that plot over by the left side of the triangle have more heavy weight congeners, and the EM2 composition matches an Aroclor 1260 pattern. The EM3 composition at the top of the triangle does not resolve a single Aroclor pattern like the other two EMs, and is shown as a bar chart fingerprint in the top of Slide 25. Since earlier crossplots with diagnostic congener pairs did not show signs of dechlorination or other common alteration patterns, fits were not attempted with possible alteration patterns and instead different ratios of Aroclors were used to try to "fit" to this endmember. As shown by the high cosine theta value of 0.98 (values closer to 1.0 indicate better fits), the best fit was obtained with a mix of 85% Aroclor 1260, 10% Aroclor 1254, and 5% Aroclor 1248. This indicates that the EM3 composition at the top corner of the PVA triangle is best represented by a mix of Aroclors, and this makes sense when discussed later in the results section. Slide 26 shows the use of coefficient of determination (CD) to assess how well model predicted values match measured values for each congener. This metric is used in PVA to help decide how many EMs are required to provide a good fit to the data. It was assumed in Slides 23 and 25 that all the data fit within a triangle and there were three EMs. But sometimes it is not clear how many endmember sources are present when looking at a datacloud of points plotted in principal component space. There are often questions about whether the data would be better fit as a line of points between two EMs, a triangle of points between three EMs, a rectangle between four EMs, or some other polygon with more EMs. So to be more objective we can plot the predicted versus measured values for each congener and inspect how the CD values increase as more EMs are added. When the appropriate number of EMs are used, most congeners reach a plateau level and further increases in EMs will not significantly increase the CD fit. In the

example shown congener PCB44 (like many of the other congeners) reaches a plateau value of about 0.9 with three EMs but does not increase significantly farther with additional EM additions. In this manner, the rather subjective practice of assuming a triangle of three end-members is sufficient to enclose the datacloud is replaced by a more objective practice of comparing CD fits of all the congeners to decide when the appropriate number of EMs has been reached. But some level of professional judgment is still required, as seen with the PCB49 plots where the CD does get better with four EMs. Several other congeners also behave like PCB49 and are the congeners more diagnostic of dechlorination so although we ruled out major amounts of dechlorination by looking at plots of specific congener pairs the PVA model may indicate there is some minor dechlorination occurring. But these small amounts will not have major impact on the proportions from the three EM solutions so for the sake of simplicity we have chosen to go with a three EM solution.

Presentation of Results

The last step in the process, the Step 6 Presentation of Results is probably the most important step in the process. Even at many sites where all the preceding steps have been done correctly, many studies fail to deliver the information in a manner that proves useful to their sites. As shown in Slide 27, many individuals responsible for making decisions at the site (RPMs with backgrounds in fields such as engineering or biology) may not follow the discussions of metrics like cosine theta or data plotted in principal component space so the results are often viewed as some type of fingerprinting “magic”. But if the fingerprinting story is built up from easy to see bar chart fingerprints to the more easily seen spatial displays like contour maps of the various EM sources (or the core diagrams, bubble plots, etc. in the following slides), then the fingerprinting becomes less magic and more easily understood and therefore acceptable to the general audience. The limitations of the study should also be presented to help understand the “robustness” of the solution at a particular site, because some sites will have a stronger fingerprinting solution than others so this should be considered when applying the results at the site.

Slide 28 shows an example of the core diagrams that can be used to present information on the spatial relationships of the EM proportions around the site. Although not shown here, this same information could be shown in 3D contours with Earthvision software in a manner similar to the RSC data in Slides 16 through 20 (although with lower sampling density the ACF data may not support contouring as well as the RSC data). On Slide 28 the results from two separate PVA runs are shown in core diagrams from an east to west transect, with Run 1 showing three cores and Run 2 showing the same three cores with the addition of two more intermediate cores. Cores have sample numbers corresponding to the earlier sampling map in Step 3, with core depth running vertically and the percent contribution from the color coded end-members (%EM) across the bottom. The subset of sample horizons that were run for ACF are shown in gray on the left of each core. The 3 proposed endmember source compositions on the right on the slide from PVA have been compared against Aroclor compositions and labeled in parentheses (as described above in Step 5). Core SB105 is from the west side of the embayment near the creek, SB079 is from the east side of the embayment near the former landfill, and SB104, SB092, and

SB94 are located between these other two cores in the same east to west transect. SB079 shows mostly contributions from EM2 which has a congener composition that closely matches Aroclor 1260. SB105 shows mostly contributions from EM3 which appears to match a mix of Aroclors 1260, 1254, and 1248. The intermediate cores appear to have a mix of all three end-members that depends on how close they are to each end of the transect. Run 2 shows similar patterns to Run 1, except that all the cores have slightly more blue EM3 contributions (indicating that in this run the sample points all plotted closer to the top corner of the PVA triangle so congener compositions were closer to EM3). By looking at results of various runs, we can see the variability, or error bars, that might be associated with using these types of core diagrams to present information about sources at the site. A core of specific interest is SB94, because it is one of the Pb210 dated cores that show the time periods when PCBs were deposited in the area. With sedimentation rates of about 1cm/yr, there was a distinct change in composition of the sources about 30 years ago (at 30 cm depth at about 1970) when EM1 decreased significantly and EM3 increased. These dated cores can help answer the “what” and “when”, but additional information is required to answer the “who” and “where”.

Additional visual displays that also provide spatial information may prove useful in showing results. Slide 29 states that although ACF can show what sources are present, additional information is required to determine where sources may originate. This additional information includes the RSC contouring maps, any upstream or upland contaminant studies that show PCB source locations, possible sediment transport data that show where sediments are coming from (or dated cores to show “when” sediments and contaminants came to the core location), and any additional site history information that might shed light on possible PCB sources. Slide 30 shows a bubble plot of the contributions of EM3 to the various surface locations around the site, where the size of the bubble is proportional to the %EM3 in the sample. Similar to the core diagrams in Slide 28, this bubble plot suggests the source for EM3 is over by the creek, and additional upstream samples show higher concentrations of PCBs are present farther up the creek. Public records show a soil cleanup done above ppm levels was completed at a land site adjacent to the creek, and this site drains directly into the creek. Additional work could be done to match the congener compositions of this site with the EM3 composition, but that is outside the scope of this project. A similar bubble plot for EM2 suggests its source is on the east side of the embayment near the former landfill. Since this landfill is on Navy property we have access to additional studies and Slide 31 shows some additional upland areas with PCBs at surface and two foot depths. An extended shoreline area in front of the former landfill shows PCB levels at 5-15 ppm, with surface sediment contours dropping off moving away from the landfill area. The congener composition of some of these upland samples is a good match to EM2, so this area represents a good candidate for the source of EM2 to the more recent surface sediments. Slide 32 provides some additional site history about the former landfill based on dated aerial photos. The shoreline fill history of the site shows all the current land area shown in the figures was originally under water prior to the 1940s when the navy acquired the property. By 1946, the red shoreline shows the bay was being filled rapidly to create useable land around the creek area. Combined Sewer Outfalls (CSOs) were located in the creek and also up behind the present location of the landfill, and while the landfill was actually being used

it was filling the former CSO channel from about 1955 to 1975. Sometime around 1970 the CSO behind the landfill was shutdown and realignment of the CSO network (which also included creating the "Moat", an enlarged underground storage capability to help prevent CSO overflows) left three CSOs in the Creek area. This 1970 time period when the CSOs were realigned corresponds to the 30 cm depth in dated core SB94, which may help explain the change in congener composition at 30 cm that might be related to this change in CSO position. So before 1970 the congener composition at depth in all cores was more similar across the whole site. Another possibility for the dramatic decrease in EM1 in more recent sediments might be related to a shoreline fill event that covered an older Aroclor 1254 source area (maybe from an even older fill event that was contaminated with Aroclor 1254 material?). It is often much harder to interpret older sources since they may have disappeared long ago, but more recent sources like those seen in surface sediments are generally easier.

Conclusions and Lessons Learned

The overall objective of this case study is to demonstrate an integrated forensics approach that the DoD can use at their sites to assist in identifying any continuing sources that need to be controlled, and assist in any apportionment of TMDL load reductions and/or cleanup costs. Slides 33 and 34 provide some conclusions from which we can learn some lessons from the case study done at Hunters Point Shipyard (HPS) that can be applied at future sites where fingerprinting may be considered. The objective of the project was not to completely solve all the fingerprinting questions at HPS, but only use this site as an example of how a forensics project could be done at similar sites. The HPS contour maps of PCB concentrations suggest two areas of very high (> ppm) levels of PCB deposition, one by the creek and one by the former landfill. The congener composition data suggest three end-members can be mixed together to produce all the patterns we see in the site samples over time, from about the 1940s time period to the present time. In the more recent sediments, it appears that an EM3 source originates over by the creek and probably represents the more recent CSO pattern. It is a mix of Aroclors 1260/1254/1248 and is most common in surface sediments of SB105. The EM2 source in surface sediments is from the shoreline area in front of the former landfill where soil levels are consistently 5-15 ppm, and they have an Aroclor 1260 composition most commonly seen at all depths in SB79. Going back in time (deeper depths in the cores) the interpretation of sources becomes more uncertain, but some patterns are clear. The shoreline area in front of the landfill was emplaced between 1960 and 1970, so before this period the location of EM2 source Aroclor 1260 pattern is not clear. There was also much more EM1 (Aroclor 1254) in the older sediments before 1970 (deeper than 30 cm in core SB94). One possibility is that older shoreline fill material had more Aroclor 1254 that acted as a source to the older sediments and later shoreline fill events covered the Aroclor 1254 source so it is not as common in recent sediments. Another possibility was the CSOs that emptied into both the creek and landfill areas were the source of the pre-1970s mix of EM1 (Aroclor 1254) and EM2 (Aroclor 1260). And after the realignment of all CSOs into the creek area the EM3 source signature appears to be coming from the creek and the EM2 source pattern is coming from the area just in front of the former landfill that was emplaced during the 1960s.

In addition to the conclusions about the PCB sources at HPS, there are general conclusions and lessons learned that can be applied to any future fingerprinting site. As stated several times in this report, a forensics study needs high quality congener data and people experienced at teasing out source relationships from these data. When there does not appear to by much alteration of PCB compositional data (as appears here at HPS), both the 18 and 44 congener datasets provide similar indications of three end-members. However, the 18 congener NOAA Status and Trends congeners may not provide enough discrimination between sources at more complicated sites with different congener mixtures. Additionally during data preparation if some congeners have too many non-detects they will need to be dropped from the dataset so an 18 congener dataset might be reduced to even fewer congeners. With the 44 congener dataset we ended up dropping 6 congeners (and a similar number of samples) from the dataset, so it is probably best to start with a dataset of more than the 18 NOAA Status and Trends congeners for a forensics study. This is especially true for sites where it is not known how much alteration may be present, since additional congeners will often be needed to discriminate these alteration patterns in addition to any Aroclor mixing. We had planned to also look at a 100 congener dataset at HPS, but with no major alteration it was not required. We anticipate comparing a 100 congener dataset at the second site where we expect more alteration (freshwater site has less sulfate reduction that interferes with dechlorination, and second site has higher PCB concentrations which should farther stimulate dechlorinating bacterial community). After also looking at a site with 100 congeners, we will offer more insight into the benefits and limitations of using different numbers of congeners at forensics sites.

The HPS site provides a clear example of how a cost-effective forensics study can be designed, especially if it is possible to leverage the study with other regulatory projects. In this case the regulatory project paid for field and analytical costs so the fingerprinting could be done for very low additional costs. At other sites there may need to be discussions of analyzing for additional congeners that are desired by the fingerprinting work, but this should be easily done with some upfront planning. One possible limitation of using existing data is care must be taken to look for inter-laboratory variability that can hide subtle patterns in the data. In the HPS case it looks like the earlier regulatory project only saw two end-members when they looked at an older 18 congener dataset compiled from multiple studies. The third endmember source EM3 was hidden by the inter-laboratory variability caused by combining different laboratory data from different studies done over different time periods. If they had looked at more congeners (such as the 44 congeners in our dataset) or had less interlaboratory variability this third endmember might have been seen.

One of the most important insights gained from the first demo was the realization of the need to develop different types of visual displays that will allow more information to be transferred to an audience of different technical backgrounds. Displays that provide spatial information of sources are often the most enlightening, starting with contour maps showing spatial relationships of the bulk concentrations. By starting the study with large numbers of cheaper RSC samples it is possible to have the data density to support 3D

contour mapping, which provides a good visual display of the site. If there is not sufficient data density to support contour mapping, other visual displays (such as bubble plots) can be used. Bubble plots are often used as visual displays of the lower density ACF data when there is not enough density to support contour mapping. Pie chart inserts on a map view of sample locations also does a good job of showing the distributions of end-members around the site. But our favorite type of display for the ACF data is probably the core diagrams shown in Slide 28 “Forensics Results” (probably due to the bias of a geochemistry background). In any case the ACF data by themselves only provide information of what sources are present, and must be viewed along with other information to determine where sources may be found. The previously mentioned RSC contour maps provide a first impression of where sources are located. By combining these data along with other site information (including site contaminant use history, other upland and upstream contaminant studies, sediment transport studies with dated cores, etc.) it may be possible to tell not only what sources are present, but where they are located and when they were placed in the sediments. Hopefully it will be possible to tell who the other PRPs might be, so the forensics study can answer all the “who, what, where, and when” questions at the site. It should be noted however, that caution needs to be exercised when presenting data with many of these visual displays. It is often possible that bias will occur when presenting some aspects of the data when all the details are not included. For example, contour maps often present a visually pleasing display but if there is not enough data density interpolations between distant samples will be highly questionable. Core diagrams shown for different PVA runs in Slide 28 show some variability depending on which samples are included in each run, so without discussion of possible error terms the exact values displayed can be questioned. As with other types of studies, forensics will perform better at some sites than others so some discussion of the limitations at a particular site should be discussed prior to applying the results for regulatory purposes.

Integrated Forensics Approach to Fingerprint PCB Sources using RSC and ACF

ER0826

Dr. Jim Leather

SPAWAR Systems Center

SERDP/ESTCP Meeting

December 3, 2009

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Slide 1. Title and POC information

Outline

- Project Team
- PCB Forensics Background
- ESTCP Project Summary
  – Show 6 step process at HPS including some metrics to assess performance objectives
- Review Results
  – Advanced Chemical Fingerprinting (ACF) determines what sources are present, and Rapid Sediment Characterization (RSC) helps show where sources are located

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Slide 2. Outline for 12/03/09 presentation

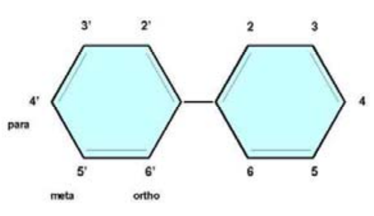
Project Team

- Jim Leather, SPAWAR – Overall Program Manager and Lead on RSC
- Greg Durell, Battelle – Lead on ACF
- Glenn Johnson, U of Utah – Lead on Statistical Methods
- Keith Forman, NAVFAC - Environmental Coordinator at Hunters Pt Shipyard
- Marc Mills, EPA ORD – Coordinator for Technology Transfer within EPA regions

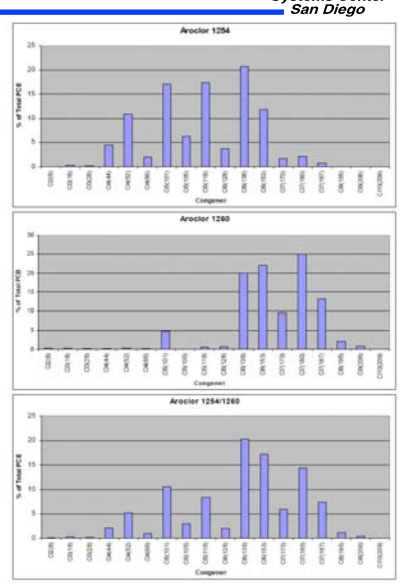
Slide 3. Project Team and general roles

PCB Background

- Polychlorinated Biphenyls (PCBs) were produced commercially by Monsanto from 1930 until 1977 under the trade name Aroclor. Each Aroclor is a different mixture of 50-100 congeners that provides a distinct fingerprint. PCBs were mainly used in transformer, hydraulic, lubricating, and cutting oils, and as additives in paints, carbonless copy paper, adhesives, plastics, and flame retardants. Their stability leads to long life in the environment, often resulting in sediment contamination.



Structure of Polychlorinated Biphenyl (PCB) Molecule



Slide 4. PCB Background and typical Aroclor mixtures

Environmental Forensics

- Objective:
  - Identification of chemical contaminant sources
- Problem:
  - Multiple sources, none characterized by unique chemical species (i.e. a tracer chemical).
  - Huge Data Sets
- Solution:
  - Multivariate Data analysis (Receptor Models) and Visualization Methods
  - See Hopke et al, 2007 for review of 25 years of EPA work on particulate metals in air, for recent review of PCBs in sediment see Johnson et al, 2002; 2007
- Multivariate Receptor Models are used to determine three parameters:
  - The number of sources (fingerprints) contributing to the system
  - The chemical composition of each fingerprint
  - The relative contribution of each fingerprint in each sample

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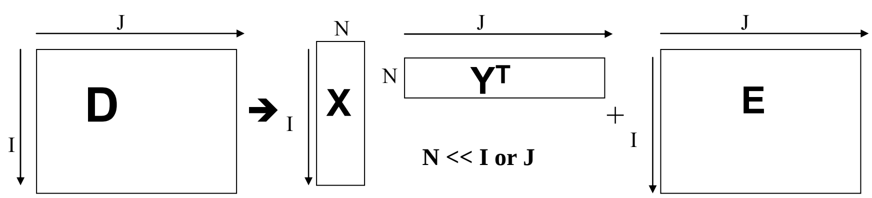
Slide 5 Introduction to Environmental Forensics

Multivariate models for environmental forensics

Principal Component Analysis (PCA) Matrix Decomposition



$$d_{ij} = \sum_{n=1}^{N} x_{in} y_{jn} + e_{ij}$$

$$\mathbf{D} = \mathbf{X}\mathbf{Y}^{\mathbf{T}} + \mathbf{E}$$

d_{ij} is the concentration of chemical contaminant j in sample i
$n=1,\ldots,N$ are a reduced number of independent environmental sources
x_{in} is the amount of source n in sample i;
y_{nj} is the amount of contaminant j in source n
e_{ij} is the remaining error term

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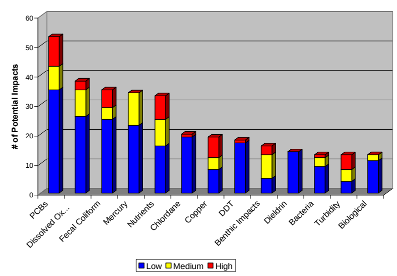
Slide 6 PCA Matrix Decomposition Figure

Problem Statement

SPAWAR Systems Center San Diego

- Navy Facilities are often the most visible and “easiest” targets among waterfront stakeholders dealing with both Cleanup and Compliance issues
- Organic Contaminants (including PCBs) and metals are major regulatory concerns, and identifying their sources is a key problem for regulatory programs
- Old standard “fingerpointing” approach to find sources of contamination often relies on finding highest concentrations and assuming nearby facility is the source
- Need a technically defensible method to fingerprint sources to not only shutoff any continuing sources, but also apportion TMDL reductions and/or cleanup costs

Figure 3. Top Impairments Based on # of Potential TMDL Impacts to Navy

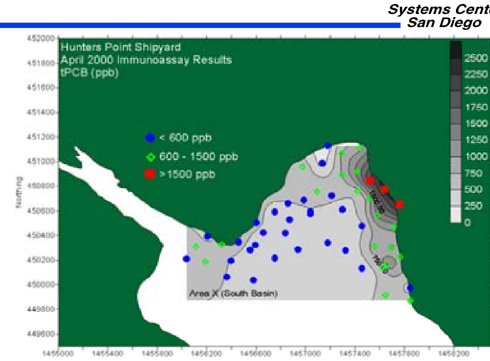


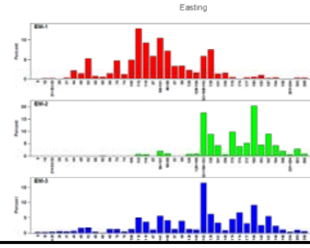
Slide 7 Problem Statement

Technical Objective

SPAWAR Systems Center San Diego

- To determine original source of PCBs to sediment, we link two components:
  - Rapid Sediment Characterization (RSC) to provide spatial and temporal coverage for cost effective concentration gradient mapping; and
  - Advanced Chemical Fingerprinting (ACF) on selected subset of samples to delineate sources using advanced laboratory methods with sophisticated data analysis and interpretation methods.





Slide 8 Technical Objective

Technology Description

SPAWAR
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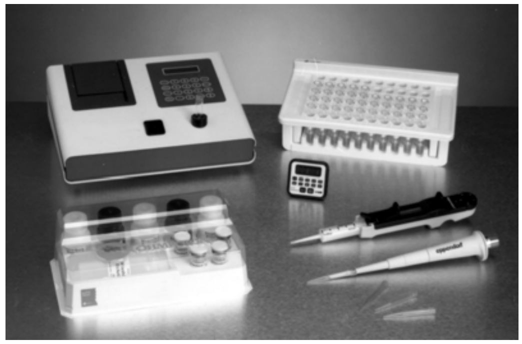
San Diego

- RSC for PCBs is done by adapting commercially available soil immunoassays for use in wet, organic-rich sediments at lower detection limits (50-100 ppb). These are acceptable modifications of EPA Method 4020 with some level of laboratory validation (10-25%).



Slide 9. RSC Technology Description

Technology Description

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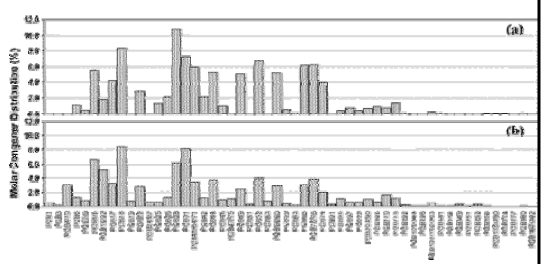
- ACF is done with EPA methods 680/1668a with modifications to detect a larger number of non-standard but diagnostic PCB congeners for differentiating potential sources. These are high resolution GC/MS methods that allow low detection limits (< 1 ppb). Laboratory data are then input into advanced statistical analysis methods (see Backup Slides) to determine number of sources and their relative contributions around the site.



–Congener profile of endmember generated by the statistical model (a) and a profile of Aroclor 1242 (b) for comparison. The cosine theta comparing these two matrixes equals 0.92.

Slide 10. ACF Technology Description

Technical Approach (Test Design)

- The project can be divided into six steps:
  - Demo site selection
  - Develop Conceptual Site Model (CSM)
  - Develop Demonstration Plan (defensible sampling)
  - Demo Rapid Sediment Characterization (RSC)
  - Demo Advanced Chemical Fingerprinting (ACF)
  - Present results in Final Report
- This user-friendly 6 step approach will be shown at Hunters Point Shipyard just south of San Francisco.

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Slide 11. Test Design Slide with Six Step Integrated Forensics Approach

Step 1 Site Selection

- Hunters Point Shipyard (HPS) is one of several BRAC sites in San Francisco Bay
- Closed to Military use in 1974
- Commercial use 1976-86
- Initial Remedial Investigation (RI) started in 1991; Final Feasibility Study (FS) completed in 2007
- PCB contamination around former landfill north of South Basin
- Large amount of RI/FS data available to leverage
- April 2008 Site Selection Memo

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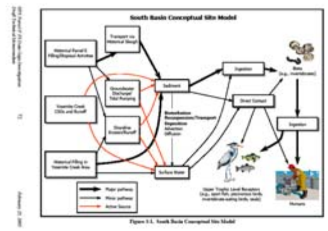
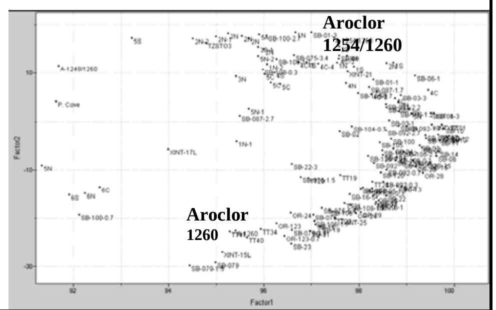
Slide 12. Step 1 Site Selection

Step 2 Develop CSM

- To develop defensible sampling strategy, Conceptual Site Model (CSM) will first develop testable hypothesis (forensic questions to be answered by Task 3 Sample and Analysis Plan).
- HPS data from multiple studies were combined to develop CSM for the regulatory project, and principal component analysis (PCA) was conducted to look for sources. A simple interpretation of this plot suggests two sources, an Aroclor 1260 source to the east by the Navy site and an Aroclor 1254 source to the west by the creek.
- But these data are only for 18 congeners, so no analysis of weathering and dechlorination was done.

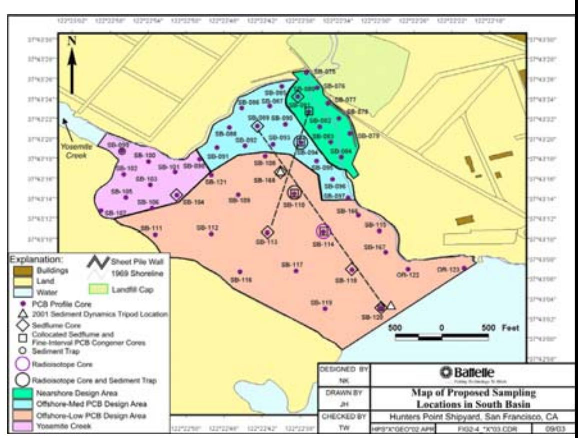




Slide 13. Step 2 Develop Conceptual Site Model (CSM)

Step 3 Sample and Analysis Plan

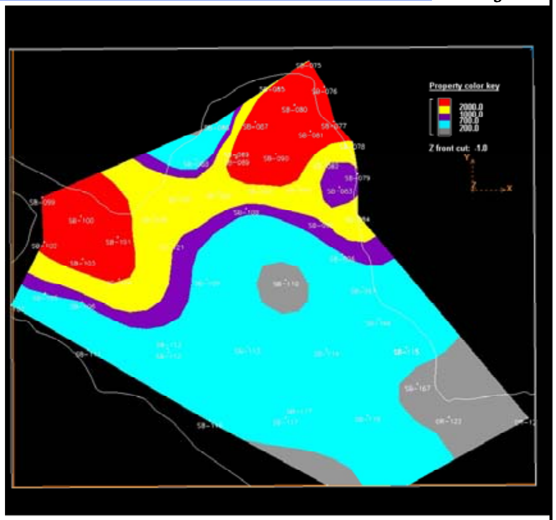
- Defensible sampling plan developed for HPS regulatory project and these analyses are used.
- Grid has higher density sampling near Navy and Creek source areas, with 7 horizons from each core for RSC (and subset for ACF with 44 congeners).
- The ESTCP Demo Plan outlines performance objectives to determine the quality of the 44 congener dataset to evaluate its use for a forensics study.



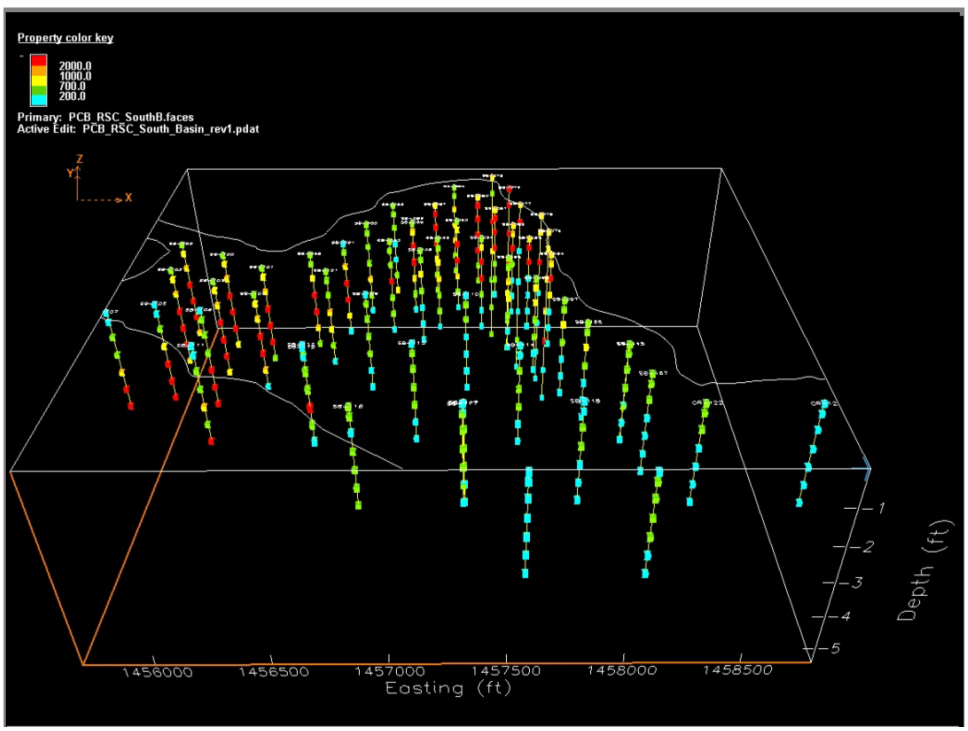
Slide 14. Step 3 Develop Sample and Analysis Plan

Step 4 RSC Demonstration

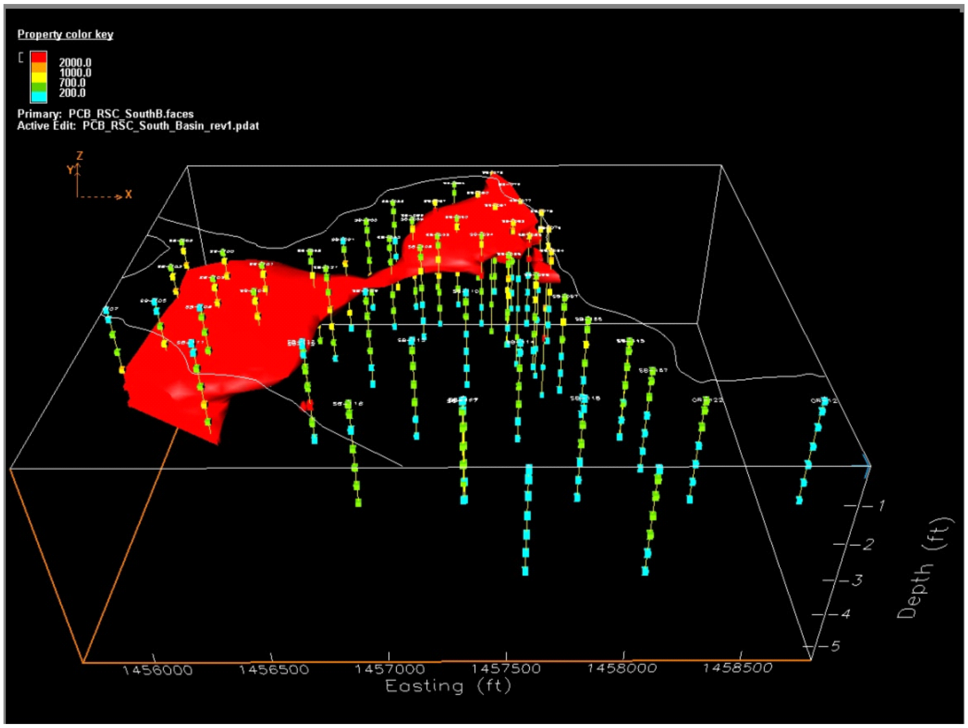
- RSC suggests two different landfill and creek source areas, better defined at depth using cores.
- RSC data provide spatial coverage for contour maps (to answer Where are sources?) and aid in the selection of ACF samples (to answer What are the sources?).
- Following contour maps can help select ACF samples to cover the spatial and concentration ranges).



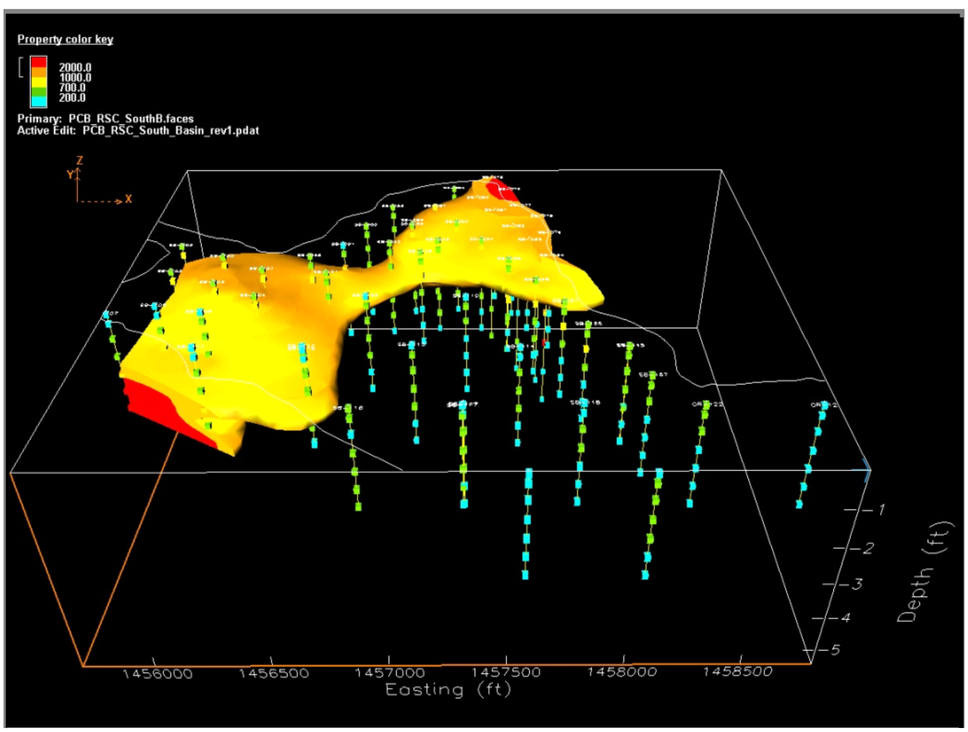
Slide 15. Step 4 RSC Demonstration



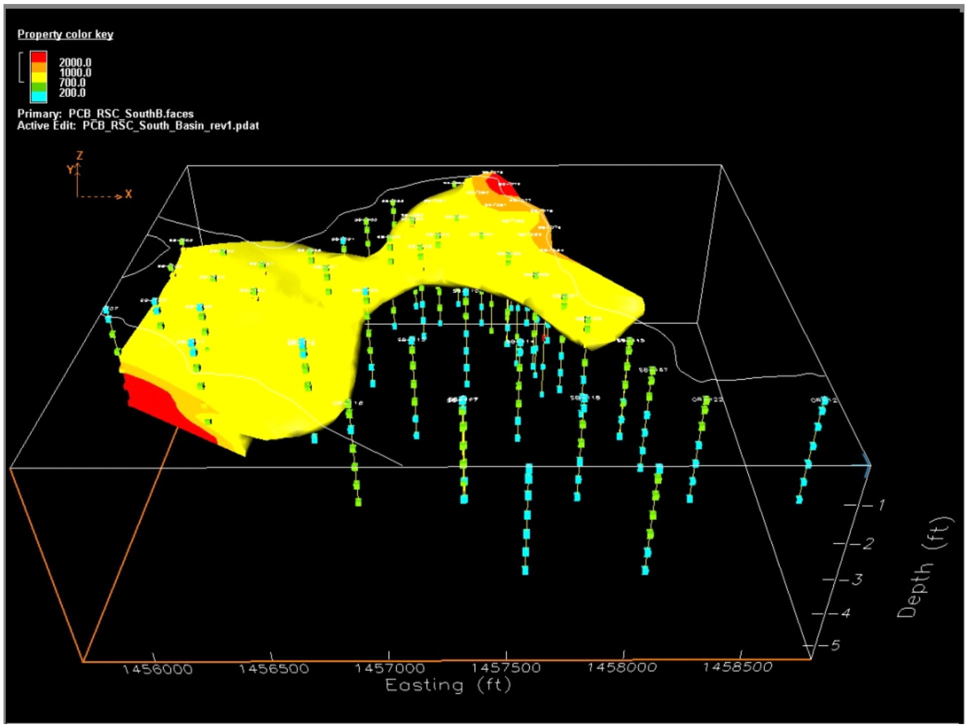
Slide 16. 3D Block diagram showing color coded sampling horizons



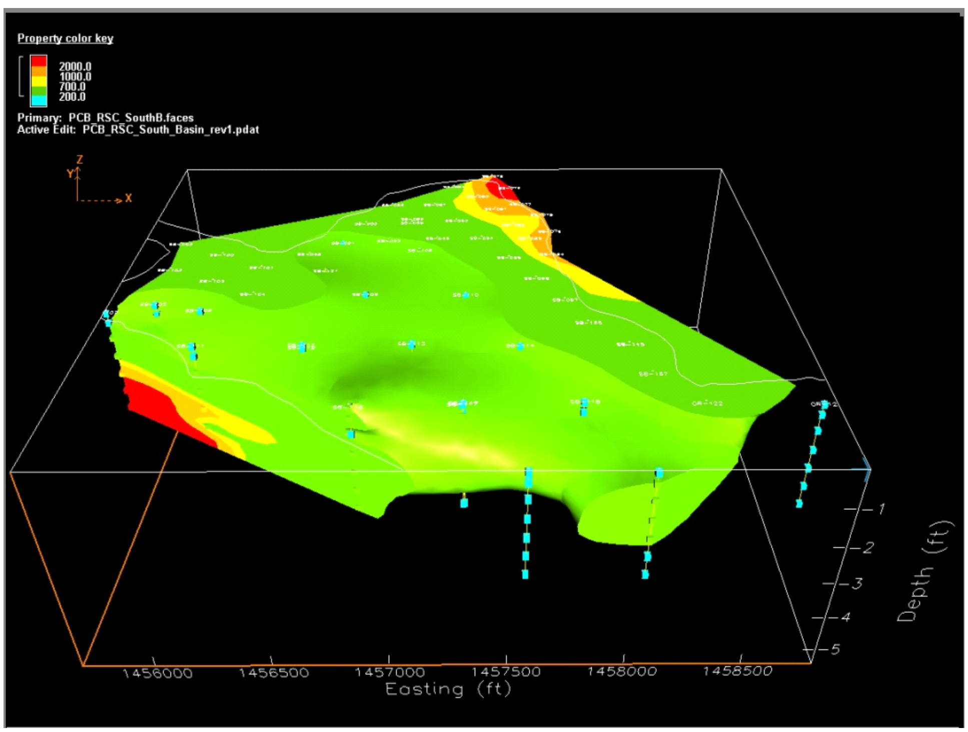
Slide 17. 3D Block diagram showing sediments above 2000 ppb.



Slide 18. 3D Block diagram showing sediments above 1000 ppb.



Slide 19. 3D Block diagram showing sediments above 700 ppb.



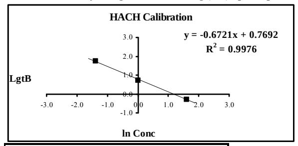
Slide 20. 3D Block diagram showing sediments above 200 ppb.

RSC Performance Metric

- PARCC used here with metrics for precision and accuracy highlighted in red.
  - Replicate runs of Sample 506 show Relative Percent Difference (RPD) of 21% (acceptable range is less than 30%).
  - Control A1254 shows a percent recovery of 107% (acceptable range is 70-130%).
- Use RSC to contour map PCB gradients and select ACF sites.

Date: 18-Dec-03

PCB ImmunoAssay Using a Direct-Reading (DR) Spectrophotometer [HACH]



conc	abs	$(r/r_0)/(1-r/r_0)$	ln Conc	LgtB
0.00	1.543			
0.25	1.311	5.65086	-1.38629	1.73
1.00	1.035	2.03740	0.00000	0.71
5.00	0.662	0.75142	1.60944	-0.29

x = ln (conc)

b = **0.7692**

m = **-0.6721**

x = (y -b) / m

$y = \ln (r/r_0)/(1-r/r_0)$

$x = ((\ln((r/r_0)/(1-(r/r_0)))-b))/m$

$conc = \exp(x) = \exp(((\ln((r/r_0)/(1-r/r_0))-b))/m)$

LABEL ID	abs	x	conc	% recov	pcb
control [ar	0.765	1.16954	3.22	107.35%	
CAD-462	0.926	0.54039	1.72		635
CAD-470	0.573	1.92770	6.87		52990
CAD-471	0.610	1.77674	5.91		9157
CAD-472	0.862	0.79379	2.21		885
CAD-474	0.899	0.64814	1.91		366
CAD-477	1.349	-1.74090	0.18		131
CAD-478	1.133	-0.36790	0.69		132
CAD-479	1.088	-0.15265	0.86		178
CAD-483	0.699	1.42494	4.16		1604
CAD-484	0.926	0.54039	1.72		5337
CAD-486	1.431	-2.64608	0.07		13
CAD-488	1.485	-3.68030	0.03		5
CAD-493	0.867	0.77422	2.17		7334
CAD-498	0.807	1.00745	2.74		8555
CAD-503 E	0.836	0.89511	2.45		1825
CAD-504 E	0.800	1.03450	2.81		3774
CAD-505 E	0.754	1.21198	3.36		6062
CAD-506 E	0.929	0.52832	1.70		3657
CAD-506	0.960	0.40240	1.50		2972
CAD-507 E	0.913	0.59245	1.81		413

RPD 20.66%

Note:

abs : measured absorbance (at λ=450 nm)

% recov : percent recovery of assay result from certified value [{IA result / cert} * 100]

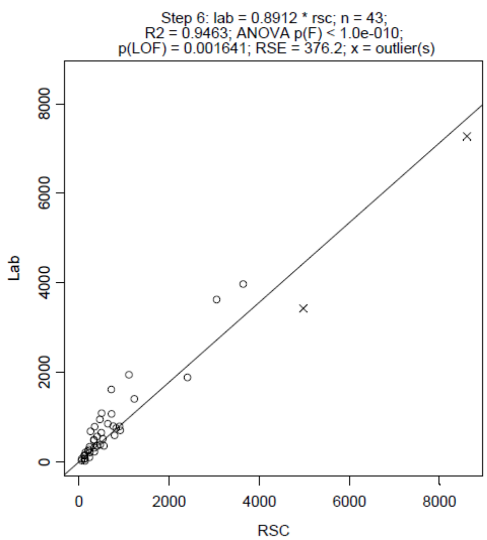
conc : analyte concentration (μg/Kg) from ImmunoAssay (IA)

pcb : dry-weight & dilution corrected analyte concentration (μg/Kg) [{ V_{MeOH} * *conc* * *df* } / *drywt*]

Slide 21. RSC Performance Metrics

RSC and ACF Comparisons

- To further validate the RSC data, comparisons were made to the ACF data (Lab=2*18 congener sum in ppb). Removal of two higher outliers would produce a better 1:1 fit and still keep R Squared (correlation coefficient) value > 0.9 indicating an excellent fit.



Slide 22. RSC Performance Metrics

Step 5 ACF Demonstration

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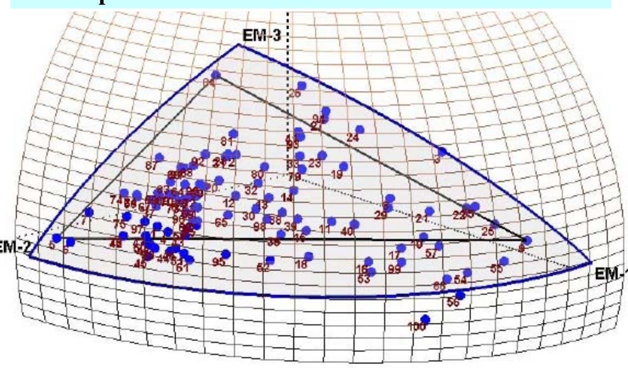
- Simple congener cross plots and principal component analysis (PCA) will be used to view compositional variations in the data.
- Polytopic Vector Analysis (PVA) and other advanced statistical techniques will be compared (see backup slides).
- Most techniques start with PCA plots where each point represents a congener fingerprint, and the more similar compositions plot closer together.

- **Principal Component Space used as Reference Space**
- **Iteratively resolves a simplex (a triangle if # sources = 3) that surrounds the data cloud.**
- **Samples are positive linear combinations of End-Members**
- **Vertices (end-members) must have non-negative compositions**



Slide 23. Step 5 ACF Demonstration

ACF Performance Metric

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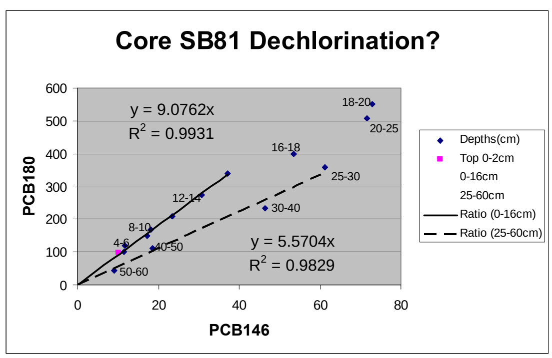
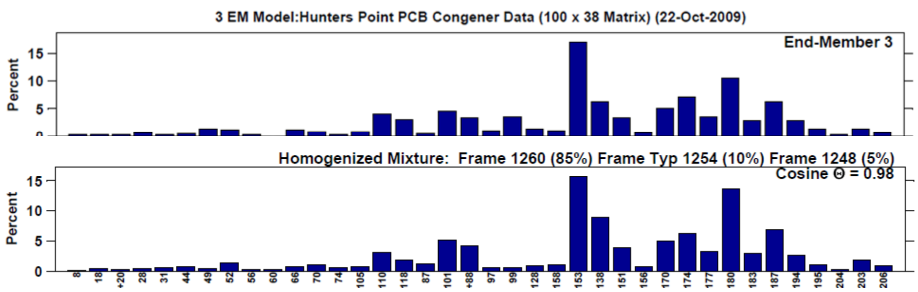
- Performance metric will be Correlation Coefficient.
- Congener cross plots should show single ratio (slope) if there is one source of PCBs.
- Multiple slopes indicate multiple potential sources, but can also indicate dechlorination.



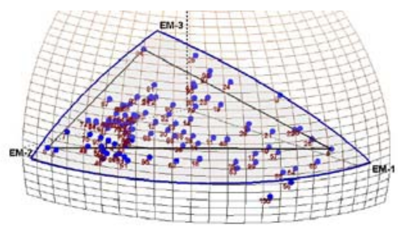
Aroclor 1260 would have a ratio of 10 and Aroclor 1254 would have a ratio of 1, so top of core with ratio of 9.1 represents a 90%/10% mixture of Aroclors 1260/1254 and bottom of core with ratio of 5.6 is a 50%/50% mixture of Aroclors 1260/1254

Slide 24. ACF Performance Metrics

ACF Performance Metric



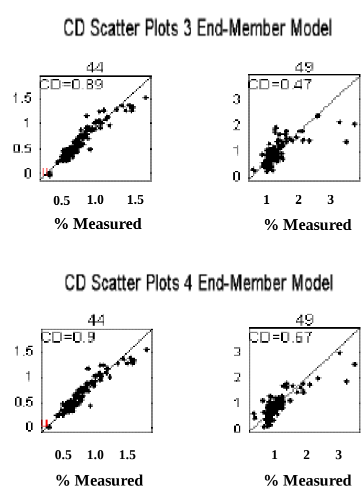
- Performance metric will be Cosine Theta.
- Congener composition can be represented by unit length vectors drawn in principal component space, with the angle between these vectors represented by Greek symbol Theta.



Slide 25. ACF Performance Metrics

ACF Performance Metric

- Performance metric will be Coefficient of Determination (CD).
- Each congener plot (here PCB44 and PCB49) shows measured value (on x-axis) versus predicted model value (on y-axis), with the CD value showing fit to the 1:1 relationship (solid line).
- Adding additional End-Members can improve CD fits but make model more complex and harder to interpret, so some degree of professional judgment is required.



Slide 26. ACF Performance Metrics

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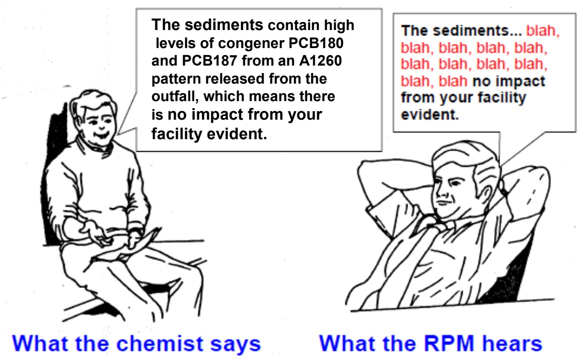
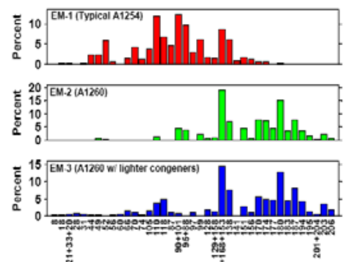
Step 6 Project Reporting





- Forensic studies need experienced operators with high quality data, but even more important may be experience with presenting results in simple terms.
- Rather than showing a graphic like the triangle of sample points plotted in 3D principal component space, we need to present data in graphical presentations that are clear to viewers with a wide range of technical expertise.

Slide 27. Step 6 Project Reporting

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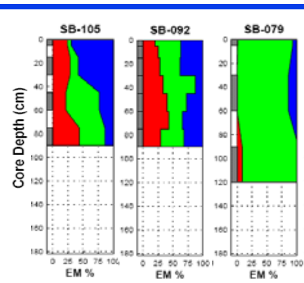
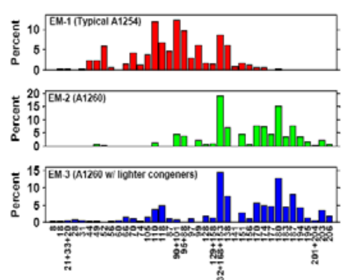
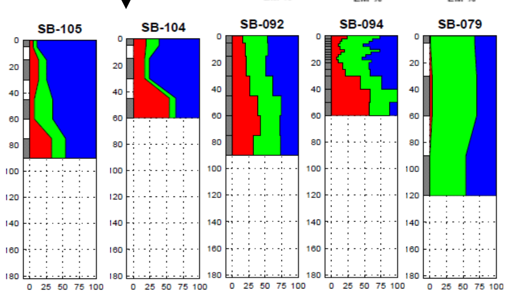
Forensic Results

Run 1





Run 2



Two different PVA runs both resolved same three end-member sources (top right). The relative contribution of each end-member (EM%) is shown in cores (top first run with three cores, bottom second run with five cores). In both runs, east side core (SB79) shows mainly EM2 (green), west side core (SB105) shows mainly EM3 (blue), and center cores show mix of potential sources.

Slide 28. Forensic Results shown in color coded core diagrams

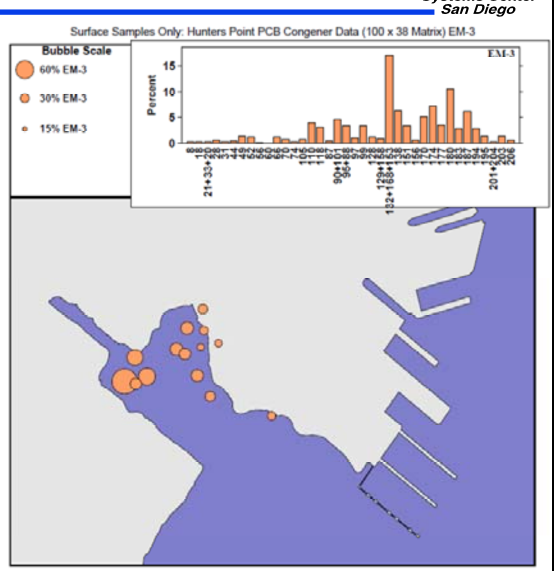
Forensic Results (con't)

- ACF provides information on what sources are present at the site.
- But additional information is required to determine where sources are located, including:
  - Higher density RSC data for concentration gradients
  - Both upstream and upland studies to find likely contaminant sources to downstream sediments.
  - Any sediment transport information, including dated cores to help determine source history.
  - Additional site history to put results into context.
- Many types of visual displays that show spatial relationships help to visualize where sources are located (contour maps, bubble plots, etc.).

Slide 29. Forensic Results (con't)

EM3 1248/1254/1260 Source

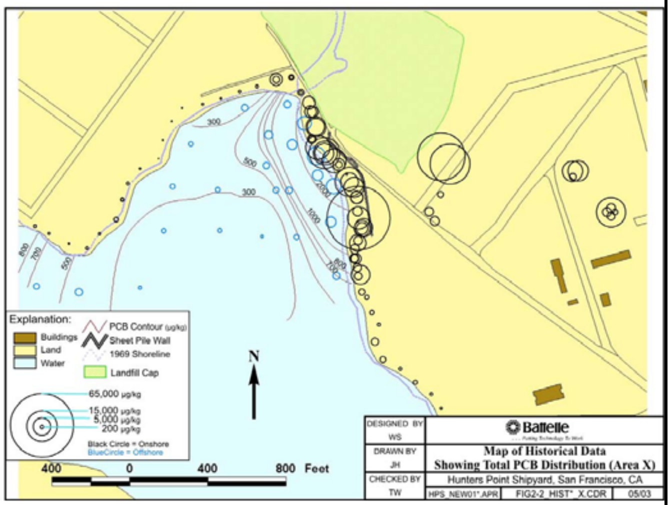
- Spatial bubble plots indicate EM3 source is located in Creek area.
- Additional upstream studies have found PCBs and one site (a drum recycling facility) has had soil cleanup at the ppm levels.



Slide 30. EM3 Source

EM2 Aroclor 1260 Source

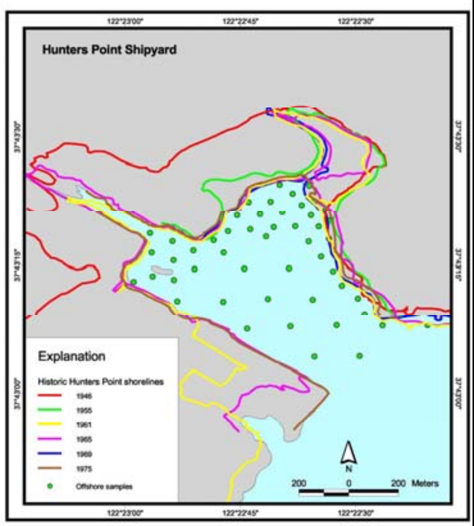
- Also need to look at ongoing shoreline study that shows elevated PCBs in the landfill area that was filled in the 1960s, with an Aroclor 1260 composition at 5-15 ppm.



Slide 31. EM2 Source

EM1 Source suggested from Site History

- Landfill area was filled over time from 1946 through 1975 where previous CSO outfall channel once existed.
- In early 1970s, the city reorganized CSOs and shutdown outfall behind landfill so all outfalls are now in the Creek area.
- Higher EM1 A1254 in cores before 1970 (deeper than 30 cm in dated SB94) from outfalls on both sides of embayment or older fill that was covered about 1970?



Slide 32. EM1 Source inferred from Site History

EM Source Summary

- Study can resolve three endmembers (EMs).
- EM3 (A1260/A1254/A1248) appears to be a source from the creek outfalls.
- EM2 (A1260) appears to be from contaminated fill along landfill shoreline.
- EM1 (A1254) appears more common at depth (prior to 1970s) and more equally mixed on both sides of South Basin. It may have come from outfalls on both sides of the Basin before they were realigned into the creek, or come from older fill events that mixed this pattern fairly evenly across the basin.

Slide 33. EM Source Summary

Forensics Summary

- Forensic studies need experienced operators with high quality data.
  - A 44 congener dataset is adequate for forensic study as long as no major dechlorination or other alteration occurs.
  - Any inter-laboratory variability can lead to differences in congener patterns that can cloud interpretations.
- But even more important may be experience with presenting results in simple terms.
  - PCA and PVA results show what PCB sources are present, but data must be presented in spatial terms and related to other lines of evidence (other upstream studies, sediment transport data, concentration gradients, etc.) to provide information on where PCB sources are located.
  - Visual presentations such as contour maps and bubble plots are easier to understand, but care must be taken to present information in a fair and accurate manner to avoid bias.

Slide 34 Forensics Summary

Backup Slides

- ◆ Background on Environmental Forensics
- ◆ Ashtabula River Site (2nd Demo)

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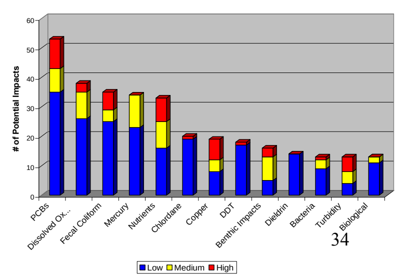
Slide 35 Backup Slides

Problem Statement

- ◆ DoD Facilities are often the most visible and "easiest" targets among waterfront stakeholders dealing with both Cleanup and Compliance issues
- ◆ Organic Contaminants (including PCBs) are a major regulatory concern, and identifing their sources is a key problem for regulatory programs
- ◆ Standard approach to find sources of contamination often relies on finding highest concentrations and assuming nearby facility is the source

Figure 3. Top Impairments Based on # of Potential TMDL Impacts to Navy



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Slide 36 Problem Statement

Technology Maturity

- RSC has been used in similar forensics approach by NAVFAC Y0817 project to fingerprint PAHs (SPAWAR Technical Report 1907). Original RSC development supported by ESTCP (Project #9707).
- ACF for PCBs using EPA Method 1668a has been done by Battelle in previous studies (see Durell refs)
- Statistical analysis methods have been used in EPA studies of metal particulates in air over the past 25 years (Hopke et al, 2006), and Johnson refs for PCBs in sediments over the past 10 years (see Johnson 2006 for review)

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Slide 37 Technology Maturity

Receptor Models

- Principal Component Analysis w/ Non-Negative Constraints (Ozeki, Christensen)
- Polytopic Vector Analysis (Imbrie, Miesch, Full, Ehrlich, Johnson)
- Alternating Least Squares (Tauler)
- Target Transformation Factor Analysis (Malinowski, Gemperline, Hopke)
- Source Apportionment by Factors with Explicit Restrictions (Lawton & Sylvestre, Henry)
- Positive Matrix Factorization (Paterro, Hopke)
- Methods Similar in:
  - Self-training (need not assume sources a priori).
  - PCA based methods (PCA: the engine under the hood).
  - Quantitative source apportionment equations by development of oblique solutions in reduced PC space
  - Non-negative constraints.

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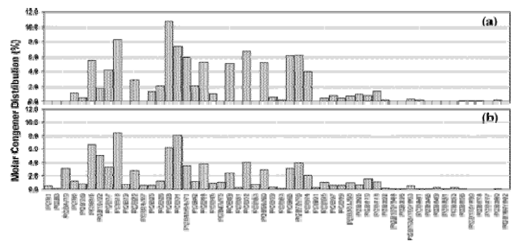
Slide 38 Receptor Models

Comparison of Methods

- PVA and other more advanced statistical techniques will be compared.
- Artificial and field datasets will be compared. Datasets with 18(NOAA S&T), 44 (Navy EcoRisk), and >100 (typical fingerprinting) congeners can be compared.



– Congener profile of endmember generated by the PVA model (a) and a profile of Aroclor 1242 (b) for comparison. The cosine theta comparing these two matrixes equals 0.92.

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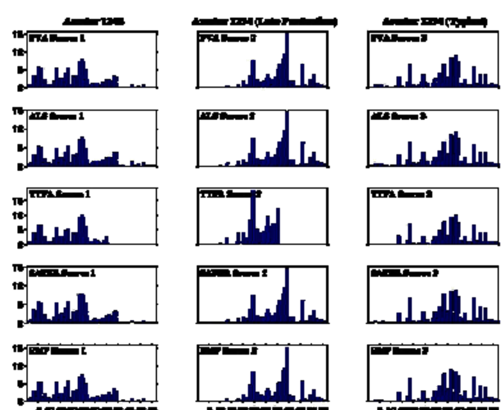
Slide 39 Comparisons of Methods

Comparison of Methods

- Comparison of five methods with artificial dataset consisting of three Aroclors. Each method (by row) shows the same three Aroclor sources (by column), indicating receptor models give similar results with simple artificial datasets. This type of comparison needs to be done with real datasets with typical imperfections (some non-detects, interferences, etc.)



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Slide 40 Comparisons of Methods

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-01-0188

1. REPORT DATE *(DD-MM-YYYY)*
December 2011

2. REPORT TYPE
Final

3. DATES COVERED *(From - To)*
September 2010 to September2011

4. TITLE AND SUBTITLE
Pollutant Source Tracking (PST) Technical Guidance

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHORS
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
SSC Pacific, 5622 Hull Street, San Diego, CA 92152–5001

8. PERFORMING ORGANIZATION REPORT NUMBER
TD 3251

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Navy's Environmental Sustainability Development to Integration

10. SPONSOR/MONITOR'S ACRONYM(S)
NESDI

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The objective of this project is to accurately quantify Navy contaminant loads by identifying, reviewing, demonstrating, and validating contaminant source tracking technologies that will provide a technical framework for Navy water program managers, enabling them to: (1) attribute existing contamination loads to support compliance programs; (2) clearly understand weaknesses, as well as how thos technologies can be used to develop management decisions for compliance ; (3) use the scientific approach and these tools to prevent arbitrary and burdensome regulatory decisions and actions that negatively impact the Navy.

This User's Guide will primarily focus on chemical fingerprinting, but will also touch on other key forensic techniques as needed.

The product will provide the Navy with a means to attribute pollution loads for compliance programs so that a significant amount of money can be saved. The Navy will be able to identify base pollutant sources, which will in turn, help identify best management practices to reduce these sources.

15. SUBJECT TERMS
contaminant loads chemical fingerprinting source tracking rapid screening technology alteration processes

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON / 19B. TELEPHONE NUMBER *(Include area code)*
U	U	U	U	168	R. George (619) 553-2776

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

SSC Pacific
San Diego, CA 92152-5001